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[Continued on next page]

(54) Title: INHIBITORS OF CORONAVIRUS

1 MFIFLLFLTLTSGSDLRCTTFDDVQAPNYTQHTSSMRGVVYPDEFIRSD
51 TLYLTQDLFLPFYSNVTGFHTINHTFGNPFVPPKDGIFYAATEKSNVVRG
101 WFGSTMNKNSQSVIIINNSTNVVIRACNPFELCDNPFPAVSKPMGTQTHY
151 MIPDNAPNCTFEYISDAPSLDVSEKSGNFKHLREBFVFNKDGPLYVYKGY
201 QPIDVVRDLPSGFNTLKPFIKPLGGINITNFRALLTAFSPAQDIWGTSA
251 AYFVGVLKPTTFMLKYDENGITITDAVDCSQNPLAELRCSVKSFEIDKGIY
301 QTSNFRVVPSCDVVRFPNITNLCPFGEVFNATKPPSVYAWERKKISNCVA
351 DYSVLYNSTPFPSEFKYGVSAKLNOLCPSNVYADSPVVKGDVVRQIAPG
401 QTGVIADYNYKLFDDFMGCVLAWNTRNIDATSTGNVNYKYRLRHGKLRP
451 FERDISNVFPSPDGKPCFPALNLCYWPLNDYGPYTTGIGYQPYRVVVL
501 FELLNA?ATVCGFKLSTDLIRKQCYNFNFNGLTGTGVLTPSSKRFQPFQ
551 FGRDVSDFMDSVRDPTSETIDISPCA?GGVSVITPGTNASSEVAVLYQD
601 VNCTDVSTAIHADQLTPAWRIYSTGNVVFQTAGCLIGAHEVDTSYECDI
651 PIGAGICASYHTVSLRSTSQRSIVAYTMSLGADSSIAYSNNTIAIPTNF
701 SISITTEVMFVSMARTSVDCNMYICGDSSTECANLLLYGSEFCTQLNRALS
751 GIAAEQRNFRVFAQVKQMYKTPILKYFGGFNFSQILPDPLK?TRRSFI
801 EDLLFNKVTLADAGFMKQYGECLGIDINARDLICAQKFNGLTVLPPLTDD
851 MIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYE
901 NQKQIANQFNKAIISQIOESLTTTSTALGKLQDVVNQNAQALNTLVKQLSS
951 NFGAIISSVLNDILSRLDKVEAEVQYDRLITGRLQSLQTYVYVQQLIRAAEI
1001 RASANLAATKMESECVLGGSKRVDFCGRGYHLMSPFQAAPHGVVFLHVTYV
1051 PSQERNFTTAPACHGKAYFPREGVVFVNGTSPWITQNRFFSPQIITTD
1101 NTFVSGNCDVIGIINNVTYDPLQPELDSFKBELDKYFKNHTSPDVLQD
1151 ISGINASVVNIQKEIDRLNEVAFKLNESLIDLQELGKYEQYIKWPNYVWL
1201 GFTAGLIAIVMTIILCCMTSCCSCLKGACSCGCKFDEDDSEPVKGV
1251 KLHYT

(57) Abstract: The present invention relates to inhibitors of coronaviruses including severe acute respiratory syndrome (SARS); methods of identifying inhibitors of coronaviruses; methods and compositions useful for therapy (treatment, or prevention) of coronavirus infections. The coronavirus inhibitors target different regions of the coronavirus Spike protein to inhibit coronavirus replication. In particular embodiments, the inhibitors include Five-Helix proteins, HR2 peptides, HR1 peptides, HR1 chimeric peptides. The present invention also features retro-inverso analogue of the coronavirus inhibitors, coronavirus inhibitors containing one or more tryptophan analogs, and / or one or more lactam bridges.

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TITLE OF THE INVENTION
INHIBITORS OF CORONAVIRUS

CROSS-REFERENCE TO RELATED APPLICATIONS

5 The present application claims benefit of U.S. provisional application 60/463,100 filed April 14, 2003, U.S. provisional application 60/467,190 filed April 30, 2003, U.S. provisional application 60/478,860 filed June 16, 2003, U.S. provisional application 60/479,429 filed June 18, 2003, and U.S. provisional application 60/479,430 filed June 18, 2003. All the applications are incorporated by reference.

10 BACKGROUND OF THE INVENTION

 Coronaviruses are nonsegmented, s/s plus strand, enveloped RNA viruses that infect a variety of mammals and birds. In humans, they cause respiratory infections, enteric infections and possibly neurological conditions. Typically, infection results in a mild, self-limited condition, such as a cold or upset stomach. Recently, there have been reports that severe acute respiratory syndrome (SARS) is caused by a coronavirus, SARS-CoV. Although most SARS victims recover, the World Health Organization estimates that SARS is fatal in about 4% of cases, particularly in individuals with an underlying condition, such as a weakened immune response, diabetes or heart disease.

 Coronavirus envelope protein generally carries two glycoproteins, M or membrane glycoprotein and S or spike protein. S protein is involved in receptor binding and cell fusion and is comprised of two subunits: the N-terminal subunit, called S1, which forms the knob-like component of the spike and the C-terminal subunit, called S2, which forms the stem of the spike. Little is known about the mechanism by which coronaviruses enter cells and presently there are no antiviral drugs consistently successful in treating any coronavirus infection, including SARS, or vaccines available to prevent infection.

25 The references cited herein are not admitted to be prior art to the claimed invention.

SUMMARY OF THE INVENTION

 This invention relates to inhibitors of coronaviruses, such as SARS-CoV; methods of identifying inhibitors of coronaviruses; methods and compositions useful for therapy (treatment, or prevention) of coronavirus infections, including methods and compositions for therapy of severe acute respiratory syndrome (SARS); and compositions for use as vaccines and immunogens and methods in which they are used to vaccinate or immunize individuals against coronaviruses, including vaccines and immunogens for use in vaccinating or immunizing individuals against SARS.

In particular embodiments, the present invention relates to C-peptides, N-peptides, Five-Helix proteins, Heptad Repeat 2 (HR2) peptides, and Heptad Repeat 1 (HR1) coiled-coil peptides, all of which target HR1 or HR2 regions of the coronavirus Spike protein to inhibit coronavirus replication. They are for use as inhibitors of coronaviruses in therapy (treatment, or prevention) of coronavirus infection, including for use as vaccines or immunogens. The exact HR1 and HR2 regions may differ from the proposed regions. However, the proposed regions provided herein are sufficient to enable the design of the inhibitors.

Reference to “comprising” and “comprises” throughout the claim indicates additional groups may be present. For example, a C-peptide comprising about 18 amino acids of SEQ ID NO: 1 may have additional groups such as amino acid regions from SEQ ID NO: 1 or other types of groups.

Reference to “peptide” in a C-peptide and N-peptide indicates amino acids joined together by a peptide bond and salt forms. The amino acids may be naturally occurring amino acids or non-naturally occurring amino acids. A non-naturally occurring amino acid (also referred to as a “modified” amino acid) contains a non-naturally occurring R-group. While “peptide” does not provide a size limitation, smaller length peptides are generally preferred. Smaller length peptides may offer advantages in ease of synthesis and solubility.

Standard one and three letter codes for amino acids are as follows: A=Ala=Alanine: C=Cys=Cysteine: D=Asp=Aspartic acid: E=Glu=Glutamic acid: F=Phe=Phenylalanine: G=Gly=Glycine: H=His=Histidine: I=Ile=Isoleucine: K=Lys=Lysine: L=Leu=Leucine: M=Met=Methionine: N=Asn=Asparagine: P=Pro=Proline: Q=Gln=Glutamine: R=Arg=Arginine: S=Ser=Serine: T=Thr=Threonine: V=Val=Valine: W=Trp=Tryptophan: and Y=Tyr=Tyrosine.

According to an embodiment of the present invention, the peptides or proteins can be modified to contain conservative substitutions. Conservative substitutions for amino acids are shown in Table 1. Factors that can be taken into account for a conservative substitution include amino acid size, charge, polarity, and hydrophobicity. (See, for example, Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-2001, Appendix 1C.) Conservative substitutions, if present, should substantially maintain or enhance either coronavirus inhibition properties or pharmacokinetic properties. In different embodiments, there are 0, 1, 2, 3, 4, 5, or 6-10 conservative substitutions.

Table 1: Conservative Substitutions

Amino Acid	Conservative Substitution
Alanine	D-Alanine, Glycine
Arginine	D-Arginine, Lysine, D-Lysine

Table 1: Conservative Substitutions

Amino Acid	Conservative Substitution
Asparagine	D-Asparagine, Glutamine, D-Glutamine
Aspartic acid	D-Aspartic acid, Glutamic acid, D-Glutamic acid
Cysteine	D-Cysteine
Glutamic acid	D-Glutamic acid, Aspartic acid, D-Aspartic acid
Glutamine	D-Glutamine, Asparagine, D-Asparagine
Histidine	D-Histidine
Isoleucine	D-Isoleucine, Valine, D-Valine, Leucine, D-Leucine
Leucine	D-Leucine, Valine, D-Valine, Isoleucine, D-Isoleucine
Lysine	D-Lysine, Ornithine, D-Ornithine,
Methionine	D-Methionine, Norleucine, D-Norleucine
Phenylalanine	D-Phenylalanine, Tyrosine, D-Tyrosine
Proline	D-Proline
Serine	D-Serine, Threonine, D-Threonine
Threonine	D-Threonine, Serine, D-Serine
Tryptophan	D-Tryptophan, Tryptophan analog*
Tyrosine	D-Tyrosine, Phenylalanine, D-Phenylalanine
Valine	D-Valine, Leucine, D-Leucine, Isoleucine, D-Isoleucine

* The tryptophan analog can be D or L.

According to an embodiment of the present invention, the peptides can be modified, e.g., containing one or more tryptophan analogs and / or one or more lactam bridges.

5 The present invention also provides retro-inverso analogues of the inhibitors of coronavirus.

As used herein, polypeptide refers to a peptide comprising about 10 to about 500 amino acids. Examples of polypeptides include C-peptides, N-peptides, the chimeric N-peptides, Five Helix, Six Helix, and their derivatives.

10 According to an embodiment of the present invention, the coronavirus inhibitor is a purified polypeptide. A "purified polypeptide" represents at least 10% of the total polypeptide present in a sample or preparation. In preferred embodiments, the purified polypeptide represents at least about 50%, at least about 75%, or at least about 95% of the total protein in a sample or preparation. Reference to "purified polypeptide" does not require that the polypeptide has undergone any purification and may include, for example, chemically synthesized polypeptide that has not been purified. According to another
15 embodiment of the present invention, the coronavirus inhibitor is a purified oligomer of polypeptide.

One aspect of the present invention describes a method for treating a patient to inhibit coronavirus replication. The method comprises the step of administering to the patient an effective amount of an inhibitor of coronavirus to the patient.

Another aspect of the present invention describes a method of inhibiting the ability of a human coronavirus to infect a cell *in vitro*. The method comprises the step of providing an inhibitor of coronavirus to a cell culture infected with the coronavirus.

Reference to "patient" indicates a mammal undergoing treatment and does not necessarily indicate the presence of a coronavirus infection. Treatment can be provided to a patient infected with the coronavirus to decrease the severity of a coronavirus infection. Treatment can also be provided prophylactically to reduce the likelihood or severity of a coronavirus infection. A patient can be a human or another type of mammal. Treatment of non-human patients may be useful in protecting pets and livestock, or in evaluating the efficacy of a particular treatment.

Reference to "effective amount" indicates an amount sufficient to provide a beneficial effect to a patient. Beneficial effects that can be achieved include detectable inhibition of viral replication or decrease in viral load. The inhibition or decrease should be sufficient to provide a medically significant decrease in the severity or likelihood of a coronavirus infection.

Other features and advantages of the present invention are apparent from the additional descriptions provided herein including the different examples. The provided examples illustrate different components and methodology useful in practicing the present invention. The examples do not limit the claimed invention. Based on the present disclosure the skilled artisan can identify and employ other components and methodology useful for practicing the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the amino acid sequence (SEQ ID NO:1) of the S or spike protein of human coronavirus recently identified in SARS victims, as well as the heptad repeats predicted by two different methods (Multicoil and Learncoil VMF). According to multicoil trimeric prediction (bold), HR1 includes amino acid residues from about 900 to about 974 of SEQ ID NO:1, while HR2 includes amino acid residues from about 1148 to about 1193 of SEQ ID NO:1. According to learncoil VMF prediction (underlined), HR1 includes amino acid residues from about 900 to about 1005 of SEQ ID NO:1, while HR2 includes amino acid residues from about 1151 to about 1185 of SEQ ID NO:1.

Figure 2 shows the amino acid sequences of C34 coil-like inhibitors, also referred to as Heptad Repeat 2 Peptides. The a, d and e residues from the heptad repeat of SARS-HR2 are grafted onto the f, b, and c positions, respectively, of the GCN4 coiled coil to make (Cys)SARS-HR2-GCN4 ((Cys)HR2G, SEQ ID NO:6). Then, through the cysteine residue, this peptide is covalently coupled to (Cys)GCN4

(SEQ ID NO:7) to form a stable dimer exposing the SARS-HR1 binding interface on one side. One example is shown for the SARS coronavirus sequence.

Figure 3 illustrates retro-inverso peptides. The parent linear peptide is shown in the middle (Boxed). The retro-inverso analog is shown in parallel (bottom) and antiparallel (top) orientation. It is apparent that in the antiparallel orientation the side-chain topology is maintained, while the direction of the peptide bond is reversed. In the retro-inverso peptide, all the amino acids are in the (D) configuration.

Figures 4A, 4B, and 4C, provide examples of tryptophan analogs.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to inhibitors of coronaviruses, such as SARS-CoV. According to an embodiment of the present invention, the inhibitors comprise sequences derived from the heptad repeat regions (e.g., HR1 or HR2) of coronaviruses.

1. The Heptad Repeat Regions of Viral Fusion Proteins

Like other enveloped viruses, coronaviruses have a viral membrane containing glycoproteins that permit the viruses to bind to target cells and achieve membrane fusion, an essential process for viral infection, by which the viral and cellular membranes combine to form a single bilayer. The S glycoprotein on the viral membranes of coronaviruses is such a fusion protein, which participates in receptor binding and membrane fusion. The S protein comprises two heptad repeat regions, HR1 and HR2, which are predicted to collapse to form a trimer of hairpin structure in the fusion process. (See, DeGroot et al. *Journal of Molecular Biology* 196:963-966 (1987); Wolf, E. et al. *Protein Science* 6:1179-1189 (1997); Singh, M. et al. *Journal of Molecular Biology* 290:1031-1041 (1999)).

Heptad repeat regions have been characterized by structural studies in membrane-fusion proteins of a variety of different viruses. In the fusion processes, the heptad-repeat sequences all form trimeric hairpin-like structures, in which a central three-stranded coiled coil is surrounded by supporting helices packed in an antiparallel manner. The trimer-of-hairpins structure is thought to bring the viral and cellular membrane together. The trimer-of-hairpins structure presumably corresponds to the fusion-active state. Extensive studies have shown that viral fusion proteins undergo a series of conformational changes to become the trimer-of-hairpins structure from a pre-hairpin intermediate conformation when exposed to the appropriate triggering signals (Hernandez et al. *Annu Rev Cell Dev Biol* 12: 627-661 (1996)). Various peptides were designed as exogenous inhibitors to bind to the pre-hairpin intermediate in a dominant-negative manner and consequently block formation of the fusion-active hairpin structures in

viruses such as HIV (Chan and Kim, *Cell* 93: 681-684 (1998); Eckert and Kim, *Annu Rev Biochem* 70: 777-810 (2001)).

The presence of the two heptad-repeat regions, HR1 and HR2, in S2 subunit of coronavirus S proteins indicates that they adopt a trimer-of-hairpins structure in their fusion process. As described herein, analysis predicts that the HR1 and HR2 regions collapse to form the trimer-of-hairpins structure consistent with the structure of HIV gp41. Peptides containing the HR1 and / or HR2 likely act in a dominant-negative manner to prevent the formation of the trimer-of-hairpins structure and inhibit infection of coronaviruses. Agents that prevent conformational changes which play a critical role in viral and cell membrane fusion, such as by stabilizing the native or intermediate states of the viral fusion proteins are expected to prevent fusion activation and block viral entry.

Applicants have analyzed the amino acid sequences of a variety of coronaviruses, including that of the recently identified human coronavirus isolated from SARS victims.

(http://ybwweb.bcgsc.ca/sars/TOR2_draft_genome_assembly_120403.fasta.gz). SARS-associated coronavirus is also described by Rota *et al.*, *Science* 300, 1394-1399 (30 May 2003); published online 1 May 2003, 10.1126/science.1085952; and Marra *et al.*, *Science* 300, 1399-1404 (30 May 2003); published online 1 May 2003, 10.1126/science.1085953.

The analysis has shown that coronavirus S protein comprises two heptad repeat regions which are predicted to form a trimer of hairpin structure. As shown in Figure 1, results of analysis of SARS spike protein (SEQ ID NO:1) by two methods (Multicoil and Learncoil VMF) identified two heptad repeat regions that are predicted to form a trimer of hairpin structure as follows.

Predicted by Multicoil, HR1 comprises amino acid residues from about 900 to about 974 of SEQ ID NO:1; and by Learncoil VMF, HR1 comprises amino acid residues from about 900 to about 1005 of SEQ ID NO:1. Predicted by Multicoil, HR2 comprises amino acid residues from about 1148 to about 1193 of SEQ ID NO:1; and by Learncoil VMF, HR2 comprises amino acid residues from about 1151 to about 1185 of SEQ ID NO:1. The composition (identity and order of amino acid residues) of the spike (S) protein of SARS coronavirus presented herein is defined with reference to the TOR2 draft genome assembly provided at the website referenced above.

Based on the understanding of the structures and functions of coronavirus Spike (S) proteins, especially their HR1 and HR2 regions, various coronavirus inhibitors are designed, produced, and tested.

Examples of the inhibitors are illustrated as follows.

2. HR2 Peptides

A subject of this invention are peptides, referred to as HR2 (heptad repeat 2) peptides, which comprise at least 20 amino acid residues from coronavirus heptad repeat 2 and are useful as inhibitors of coronavirus infection of mammalian cells, such as human epithelial cells. Such peptides are also referred to as C-peptides, because the S2 subunit is at the C terminus of coronavirus S protein and the HR2 region is C-terminal of the HR1 region (Fig. 1). HR2 peptides are thought to bind to HR1 region in the pre-hairpin intermediate of S protein in a dominant-negative manner, block formation of the fusion-active hairpin structures, and consequently inhibit infection of coronaviruses such as SARS-CoV.

HR2 peptides comprise from about 20 to about 60 amino acid residues from the predicted HR2 region. In some embodiments, HR2 peptides comprise from about 20 to about 50 amino acid residues from the predicted HR2 region. Amino acid residues present in these HR2 peptides may be contiguous amino acid residues that occur sequentially in a coronavirus HR2 or may be noncontiguous amino acid residues that are separated by intervening amino acid residues in the coronavirus HR2. In the embodiments of all peptides described herein in which noncontiguous amino acid residues comprise the peptides, amino acid residues other than those that occur between the noncontiguous residues in the coronavirus (e.g., other than those that occur between the noncontiguous amino acid residues in SARS in nature) are introduced in order to maintain, for example, appropriate distance between the residues and ensure that the resulting peptide has an appropriate conformation. An example of the noncontiguous amino acid residues is shown in Fig. 2 and section 3 as follows.

Specific embodiments of SARS HR2 peptides include any combination of from about 20 amino acid residues to about 60 amino acid residues of SARS HR2 peptide, homologues and variants of such sequences, including variants that result from correction of any errors in the TOR2 draft genome assembly published at http://ybweb.bcgsc.ca/sars/TOR2_draft_genome_assembly_120403.fasta.gz and additional SARS HR2 regions encoded by the genome of additional coronaviruses identified or obtained from SARS victims that differ from the referenced publicly available sequence.

In specific embodiments, SARS HR2 peptides comprise from about 20 to about 60 amino acid residues, which can be contiguous or noncontiguous in the region as it occurs in SARS virus, of the following sequence, which is residues 1144-1200 or 1142-1193 of the predicted spike protein of SARS Tor2 (SEQ ID NO:1).

Amino acid residues 1144-1200 SEQ ID NO:1 has the sequence as follows:
 PDVDLGDISGINASV¹VNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYVWL (SEQ ID NO:2), in which solvent exposed regions of the HR2 include the b, c and f positions of the heptad repeat. The solvent exposed positions are underlined.

Additional peptides can be identified using known methods, such as by determining the location and composition (amino acid sequence) or heptad repeat 2 and selecting peptides of from about 20 to about 60 amino acid residues. Examples of such HR2 peptides are partial or full sequence of SEQ ID NO:2. HR2 peptides can consist of amino acid residues from N to (N+M-1) of SEQ ID NO:2, wherein M is the length of the HR2 peptide, and N can be any integral number from 1 to (58-M).

In specific embodiments, HR2 peptides consist of amino acid residues from 5 to 50, from 9 to 41, from 21 to 57, from 5 to 41, from 30 to 57, or from 1 to 57 of SEQ ID NO:2. The region of residues from 30 to 57 of SEQ ID NO:2 appears to be very highly conserved among coronaviruses and, thus is a peptide of particular interest for designing or identifying C-peptide/HR2 peptide inhibitors.

According to a preferred embodiment of the present invention, the HR2 peptide has a sequence of residues 1148-1182 or 1148-1185 of SEQ ID NO:1. Residues 1148-1182 of SEQ ID NO:1 has the sequence as follows: LGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL (SEQ ID NO:3).

3. HR2 Coil-Like Inhibitors

A further subject of this invention are coronavirus inhibitors, referred to as HR2 coil-like inhibitors, which are stable dimers that expose an HR1 binding site in such a manner that it is available for binding. HR2 coil-like inhibitors are thought to inhibit infection of coronaviruses such as SARS-CoV with the same mechanism as that of HR2 peptides.

In this embodiment, amino acid residues a, d, and e of coronavirus HR2 (e.g., SARS-CoV-HR2) are grafted onto the f, b and c positions, respectively, of a coiled coil, such as the GCN4 coiled coil GCN4-p1, to produce (Cys)-HR2-coiled coil (e.g., (Cys)-SARS-CoV-HR2-GCN4). This peptide is then covalently coupled to (Cys) coiled coil (e.g., (Cys)GCN4) to form a stable dimer exposing the HR1 binding interface of the coronavirus (here, the SARS-CoV-HR1 binding interface) on one side. Examples of such HR2 coil-like inhibitors are shown in Figure 2. GCN4-p1 is a dimeric coiled coil whose sequence was derived from GCN4, a yeast transcription activator (O'Shea, E. K., et al, *Science* 254:539-544 (1991)).

Additional coil-like inhibitors can be identified using similar techniques, which rely on identification of the heptad register, followed by application to the repeat sequence of one or more coil prediction programs (e.g., Multicoil, Learncoil VMF) and grafting of amino acid residues onto an appropriate coiled coil. See, for example, Sia, S. Doctoral Thesis entitled Rational Design of peptide inhibitors of HIV-1 entry. December 2001, submitted to the Committee on Higher Degrees in Biophysics in partial fulfillment of the requirements of Doctor of Philosophy in the subject of Biophysics, Harvard University (see also *Proc Natl Acad Sci USA*;100:9756-61 (2003)).

4. HR1 Peptides

HR1 (Heptad Repeat 1) peptides or inhibitors, also referred to as N-peptide inhibitors, are a further subject of this invention. HR1 peptides are thought to bind to HR2 region in the pre-hairpin intermediate of S protein in a dominant-negative manner, block formation of the fusion-active hairpin structures, and consequently inhibit infection of coronaviruses such as SARS-CoV.

Such HR1 peptides generally include from about 28 to about 60 amino acid residues from coronavirus HR1 peptide, although in some cases, they may include fewer than 28 or more than 60 HR1 amino acid residues. The amino acid residues in the HR1-peptide inhibitors can be residues that occur sequentially (are contiguous) or nonsequentially (are noncontiguous) in the coronavirus HR1 as it occurs in nature. The terms Contiguous and noncontiguous and sequential and nonsequential are used with reference to the amino acid sequence of a peptide or protein (e.g., HR1, HR2) as it occurs in the virus in nature.

A wide variety of HR1 peptides can be identified and produced, using known methods and the present invention encompasses all HR1 peptides from about 28 to about 60 or about 80 amino acid residues from the HR1 repeats described herein. In particular embodiments, HR1 peptides of the present invention include all peptides of from about 28 to about 60 or about 80 amino acid residues of SARS coronavirus HR1, defined with reference to the deduced amino acid sequence encoded by the referenced publicly available SARS genome data (SEQ ID NO:1 and Fig. 1). In addition, the amino acid sequences of HR1 peptides can be altered, such as by addition, substitution or deletion of one or more amino acid residues, in order to alter certain characteristics (e.g., to increase peptide stability, enhance peptide solubility).

HR1 peptides can have a length of from about 28 to about 60 or about 80 amino acid residues, and be derived from residues 900-1005 or 889-972 of SEQ ID NO:1. Examples of HR1 peptides include, but are not limited to: A peptide having a sequence of residues 896-972, 900-938, 914-949, 922-956, 943-980 or 943-981 of SEQ ID NO:1.

Another embodiment of this invention is a complex of an HR1-peptide inhibitor tethered (with an appropriate linker, which can be made of amino acid residues, or can be a chemical or synthetic linker) to a component, such as a ligand (protein or small molecule) that binds coronavirus envelope protein. The complex is useful as a therapeutic agent, because the ligand will increase the effective concentration of the tethered HR1-peptide inhibitor moiety in the vicinity of the coronavirus envelope protein.

HR1 peptides are capable of forming trimeric coiled coil. The HR1 trimeric coiled coil could be stabilized by including cysteine residues to form interchain disulfide bonds in a chemically synthesized or recombinant peptide greater than or equal to 28 and less than or equal to 60 or 80 sequential residues from

the predicted HR1 region. The recombinant versions could contain helix capping residues on the N- and C-terminus. Possible capping residues for the N-terminus are T, D and S. Possible capping residues for the C-terminus are G, R, H, N, or K. Possible amino-terminal caps for the synthetic peptides are acetyl (Ac) or succinimide (suc). For SARS virus, the HR1 comprises the sequence of residues 900-1005.

5 Possible inhibitors of SARS-CoV include, but are not limited to:

CCGTTTSTALGKLDVVNQNAQALNTLVKQLSSNFGAIS (SEQ ID NO:8),

CCGENQKQIANQFNKAISQIQESLTTTSTALG (SEQ ID NO:9), and

CCGALNTLVKQLSSNFGAISSVLNDILSRDKVEAEVQIDR (SEQ ID NO:10).

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5. HR1 Chimeric Peptides

The present invention also relates to HR1 chimeric peptides comprised of an HR1 peptide fused to a peptide that is capable of forming a solubilizing trimeric coiled coil. The HR1 chimeric peptides form stable and soluble HR1 chimeric trimeric coiled coil. HR1 chimeric peptides are thought to inhibit infection of coronaviruses such as SARS-CoV with the same mechanism as that of HR1 peptides.

15 Examples of the HR1 chimeric peptides include HR1-IQ peptides and HR1-IZ peptides.

5.1. The HR1 peptide portion of the HR1 chimeric peptides

According to an embodiment of the present invention, a HR1 chimeric peptide comprises at least a portion of HR1 of coronavirus S2 protein, as disclosed in sections 1 and 4. The HR1 region component of these peptides can be of varying length and will typically be from about 15 to about 50 amino acid residues from the coronavirus HR1 region. The HR1 peptide, in some cases, may be shorter than 15 amino acid residues or longer than 50 amino acid residues.

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5.2. The peptides capable of forming solubilizing trimeric coiled coils

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The solubilizing trimeric coiled-coil peptide can be from (comprise amino acid residues that correspond to those in) a variety of sources, including IQ peptides and IZ peptides.

IQ peptide can be from (comprise amino acid residues that correspond to those in) a variety of sources, including yeast transcription activator GCN4, such as GCN4-pII or GCN4-pIQI; Moloney Murine Leukemia Virus (MoMLV); and the ABC heterotrimer.

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The amino acid residues that comprise an IQ peptide of the present invention can be amino acid residues that are sequential (consecutive) or noncontiguous (nonconsecutive) in the trimeric coiled-coil peptide from which it is derived and/or amino acid residues that are sequential (consecutive) or noncontiguous (nonconsecutive) in HR1 peptide, provided that the resulting IQ peptide (the IQ peptide in

which they are present) is stable, soluble, helical, and trimeric under physiological conditions. In the embodiments of IQ peptides in which nonconsecutive amino acid residues of either or both components of the IQ peptide are present, the residues, as included in the IQ peptide, can be consecutive or can be separated or joined by a linker. The linker can be, for example, an amino acid residue(s) that does not occur between two amino acid residues in the peptide from which the component is derived. Alternatively, the "linker" can be a chemical or synthetic linker.

A component of an IQ peptide or HR1-IQ peptide of the present invention is considered to be derived from another peptide (e.g., a trimeric coiled coil or HR1 of a coronavirus) if the component itself (or the nucleic acid molecule(s) that encode the amino acid sequence) is obtained or isolated/separated from a source in which it occurs (e.g., from a cell in which the peptide occurs, such as a portion of a protein from which it can be removed) or is produced by recombinant DNA methods, chemical synthesis or any other method, to comprise an amino acid sequence or a nucleic acid sequence that is the same as or substantially the same as the sequences of the peptide. That is, the term is intended to be interpreted broadly and does not require that a component be physically derived from the peptide referred to.

The trimeric coiled coil peptide component of an IQ peptide must be sufficient in amino acid composition (identity and number/length) to result, when joined to the HR1 peptide portion, in formation of a soluble trimeric helical (coiled-coil) IQ peptide.

In the embodiments in which the soluble IQ peptides comprise an IQ region that is a GCN4 trimeric coiled-coil peptide, they are referred to as IQN peptides. As discussed in section 3, GCN4-p1 is a dimeric coiled coil whose sequence was derived from GCN4, a yeast transcription activator (O'Shea, E. K., et al, *Science* 254:539-544 (1991)). Based on GCN4-p1, trimeric coiled-coil peptides have been developed, such as GCN4-pII (Harbury, P. B., et al, *Nature* 371:80-3 (1994)) and GCN4-pIQI (Eckert D.M., et al, *J. Mol. Biol.* 284:859-65 (1998)). The trimeric coiled-coil peptides can be used to fuse with the HR1 sequence.

The chimeric HR1-IQN peptides comprise all or a portion of GCN4-pIQ I or a modified version of all or a portion of GCN4-pIQI, such as a modified portion that includes mutations for increased solubility, and an HR1 peptide of from about 15 to about 50 amino acid residues.

In certain embodiments of the IQN peptides of the present invention, the trimeric coiled-coil peptide, referred to as the "GCN4 portion", comprises at least 15, 16, 17, 18, 19 or 20 amino acid residues of GCN4 (S. K. Sia and P. S. Kim, *Proc. Natl. Acad. Sci. U.S.A.* 100:9756-9761 (2003)). The amino acid residues present in the components of an IQN peptide can correspond to amino acid residues that are sequential (consecutive) or nonsequential (nonconsecutive) in, respectively, the GCN4 transcription activator (or GCN4-pIQI) and HR1 peptide or a modified version of the activator or the HR1 peptide, provided that the resulting IQN peptide is an inhibitor of coronavirus infection of cells, as described

herein. The IQ and IZ peptides of the present invention can be produced as a continuous peptide or as components that are joined or linked after they are formed. As used herein, the terms "joined" or "joined in such a manner" or "incorporated" include incorporating amino acid residues by either approach.

For example, the GCN component of an IQN peptide can comprise consecutive amino acid residues from GCN4-pIQI, modified, if desired (e.g., to increase solubility). Alternatively, amino acid residues that are not consecutive in the GCN4 activator (or in GCN4-pIQI), joined in such a manner that they are nonconsecutive or consecutive in the resulting GCN4 component of an IQN peptide, can be incorporated in the IQN peptide. Similarly, the amino acid residues of the HR1 peptide component of an IQN peptide of the present invention can be amino acid residues that occur consecutively or nonconsecutively in HR1 peptide of SARS S protein and can be incorporated into an IQN peptide in such a manner that they are consecutive or nonconsecutive in the resulting peptide. In the embodiments in which nonconsecutive amino acid residues are used, they can be separated by one or more "linker" molecules, if needed to retain the respective functions/characteristics of the components and of the IQN peptide. For example, an amino acid residue(s) other than the residue(s) that normally occur between two amino acid residues of GCN4 or HR1 peptide can be used to link or join the two amino acid residues in the IQN peptide. Alternatively, the linker can be a chemical or synthetic linker, for example. IQN36, as well as versions of IQN17 that are shortened in the 'IQ' region, are also described. These shortened versions may be therapeutically advantageous because, for example, they are easier and less expensive to produce than are larger peptides.

The examples of IQ peptides include:

GCN4-pIQI: RMKQIEDKIEEILSKQYHIENEIARIKKLIGER (SEQ ID NO:11)

GCN4-pIQI': RMKQIEDKIEEIESKQKKIENEIARIKKLIGERY (SEQ ID NO:12)

A specific embodiment of an IQN peptide is IQN17, which contains 29 residues of GCN4-pIQI, including three mutations for increased solubility. The sequence of IQN17 consists of residues 1-29 of SEQ ID NO:12.

Shortened versions of IQN17, which each include a shorter GCN component than is present in IQN17, are also the subject of this invention. Specific examples of these shortened IQN17 peptides are:

- a) shortened IQN17 #1, consisting of residues 22-29 of SEQ ID NO:12;
- b) shortened IQN17 #2, consisting of residues 15-29 of SEQ ID NO:12;
- c) shortened IQN17 #4, consisting of residues 8-29 of SEQ ID NO:12;
- d) shortened IQN17 #5, consisting of residues 8-28 of SEQ ID NO:12; and
- e) shortened IQN17 #3, in which there are 15 non-HR1 amino acid residues KIKKIENEIARIKKL (SEQ ID NO:13). This is GCN4-pIQI' with a Q to I mutation, and is referred to as GCN4-pII'.

The solubilizing trimeric coiled-coil peptide can also be from other designed trimeric coiled coils, such as the isoleucine zipper (IZ) described by Tanaka et al. (Suzuki, K., Hiroaki, H., Kohda, D. & Tanaka, T. *Protein Eng.* 11:1051–1055 (1998)), or derivatives of this 'IZ' sequence. In those embodiments in which the trimeric coiled-coil peptide is from the isoleucine zipper, they are referred to as IZ peptides.

Tanaka's isoleucine zipper: Ac-YGGIEKKIEAIEKKIEAIEKKIEAIEKKIEA-NH₂ (SEQ ID NO:14)

The "IZ" molecule derivative: Ac-YGGIKKEIEAIEKKIEAIEKKIEAIEKKIEA-NH₂ (SEQ ID NO:15).

Moreover, the solubilizing trimeric coiled-coil peptide can comprise a trimeric coiled-coil peptide from HIV.

5.3. HR1 chimeric peptide configurations

In one embodiment, the components of the HR1-IQ peptides are present in the following "order": N-terminus--trimeric coiled-coil peptide--HR1 peptide-- C-terminus. Alternatively, the order of components can be reversed: N-terminus-HR1 peptide-trimeric coiled-coil peptide-C-terminus.

HR1-IQN17 and other HR1-IQN peptides can be tested for their ability to inhibit coronavirus infection of cells using known methods.

Soluble HR1 chimeric peptides of the present invention comprise, in specific embodiments, the amino acid sequences shown as follows:

TIKKEIEAIEKKIEAIEKKIEAIEKKIEAIEKKIEA-NH₂ (SEQ ID

NO:16), or a peptide consisting of residues 2-63 of SEQ ID NO:16;

TRMKQIEDKIEEIESKQKKIENEIARIKKLITTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISG

(SEQ ID NO:17), or a peptide consisting of residues 2-67 of SEQ ID NO:17;

RMKQIEDKIEEIESKQKKIENEIARIKKLISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLS (SEQ ID NO:18);

RMKQIEDKIEEIESKQKKIENEIARIKKLIENQKQIANQFNKAISQIQESLTTTSTALG (SEQ ID NO:19);

TRMKQIEDKIEEIESKQKKIENEIARIKKLIENQKQIANQFNKAISQIQESLTTTSTALG (SEQ ID NO:20);

TIKKEIEAIEKKIEAIEKKIEAIEKKIEAIEKKIEA-NH₂ (SEQ ID NO:21), or

a peptide consisting of residues 2-56 of SEQ ID NO:21;

TIKKEIEAIEKKIEAIEKKIEAIEKKIEAIEKKIEA-NH₂ (SEQ ID NO:22), or a peptide consisting of residues 2-49 of SEQ ID NO:22;

TRMKQIEDKIEEIESKQKKIENEIARIKKRLQSLQTYVTQQLIRAAEIRASAN (SEQ ID NO:23), or a peptide consisting of residues 2-53 of SEQ ID NO:23;

TRMKQIEDKIEEIESKQKKIENEIARIKKALNTLVKQLSSNFGAISSVLNDILSRDKVEAEVQIDR (SEQ ID NO:24), or a peptide consisting of residues 2-67 of SEQ ID NO:24;

5 TIKKEIEAIKKEQEAIKKKIEAIEKALNTLVKQLSSNFGAISSVLNDILSRDKVEAEVQIDR (SEQ ID NO:25), or a peptide consisting of residues 2-63 of SEQ ID NO:25.

IKKEIEAIKKEQEAIKKKIEAIEKEISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLS (SEQ ID NO:26); and

IKKEIEAIKKEQEAIKKKIEAINVLYENQKQIANQFNKAISQIQESLTTTSTALGKLQ (SEQ ID NO:27).

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Examples of IZ peptides, referred to as IZN36, IZN23 and IZN17-like inhibitors of SARS coronavirus are shown as follows.

IKKEIEAIKKEQEAIKKKIEAIEKEIENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVN (SEQ ID NO:28), or a peptide consisting of residues 1-49 of SEQ ID NO:28;

15 IKKEIEAIKKEQEAIKKKIEAIEKEINQNAQALNTLVKQLSSNFGAISSVLNDILSRDKVE (SEQ ID NO:29), or a peptide consisting of residues 1-49 of SEQ ID NO:29;

IKKEIEAIKKEQEAIKKKIEAIEKSLTTTSTALGKLQDVVN (SEQ ID NO:30);

IKKEIEAIKKEQEAIKKKIEAIEKAISSVLNDILSRDKVE (SEQ ID NO:31);

20 SQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSSIKKEIEAIKKEQEAIKKKIEAIEKEIG (SEQ ID NO:32)

Such inhibitor peptides can be administered to an individual, such as in a pharmaceutical composition comprising one or more (at least one) of the IZ peptides and an appropriate carrier (e.g., an appropriate buffer or carrier) to prevent or inhibit SARS infection in the individual.

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6. Five-Helix Proteins

Another embodiment of the present invention encompasses Five-Helix or Five-Helix proteins. The Five-Helix proteins comprise three heptad repeat 1 (HR1) components and at least two and up to, but not including, three heptad repeat 2 (HR2) components of coronavirus S2 protein, and are soluble under physiological conditions. The Five-Helix proteins interact with or bind to HR2 of coronavirus S2 protein; and inhibit coronavirus infection of mammalian cells, particularly human cells, such as epithelial cells of the upper respiratory tract or gastrointestinal tract. Thus, Five-Helix includes three HR1 components and at least two HR2 components of coronavirus S2 protein. It can also include a portion of a third

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coronavirus S2 HR2, but does not comprise three entire S2 protein HR2 components. In each case, the HR1 and HR2 components are separated by linkers, preferably amino acid residue linkers, which can be of any length. The amino acid linkers can be, for example, GGGSSGGGSGG (SEQ ID NO:33).

Also the subject of the present invention is Six-Helix protein, which comprises three HR1 components and three HR2 components of coronavirus S protein, joined by linkers, such as amino acid residue linkers. In other embodiments, the amino acid sequence of Six-Helix differs from those shown in the figure by addition, deletion, substitution or alteration of at least one amino acid residue. Six-Helix protein is useful not only for producing Five-Helix, but also as a negative control in screening for drugs that inhibit coronavirus infection of cells.

In one embodiment, a Five-Helix protein of this invention comprises three heptad repeat 1 (HR1) components and at least two and up to, but not including, three heptad repeat 2 (HR2) components of an S2 protein of a coronavirus that causes SARS; interacts with or binds to HR2 of the SARS-CoV S2 protein; and inhibits infection of human cells, such as infection of cells of the epithelium of the respiratory tract.

In one embodiment, Five-Helix can be represented as: HR1-linker-HR2-linker-HR1-linker-HR2-linker-HR1, wherein HR1 represents a coronavirus HR1 and HR2 represents a coronavirus HR2. In a further embodiment, Five-Helix can be represented as: HR1-linker-HR2-linker-HR1-linker-HR2-linker-HR1-linker-partial HR2 wherein HR1 represents a coronavirus HR1 and HR2 represents a coronavirus HR2 and "partial HR2" indicates that this component is a portion of a coronavirus HR2. The term Five-Helix or Five-Helix protein encompasses all such embodiments (those including three HR1 components and two or more, but less than three, HR2 components, separated by appropriate linkers). In specific embodiments, the HR1 and HR2 components are human coronavirus heptad repeats and partial HR2 is a partial (less than a complete) human coronavirus heptad repeat.

In further specific embodiments, the Five Helix protein can be represented as: HR1 of SARS virus-linker-HR2 of SARS virus-linker-HR1 of SARS virus-linker-HR2 of SARS virus-linker-HR1 of SARS. In further specific embodiments, the Five Helix protein can be represented as: HR1 of SARS virus-linker-HR2 of SARS virus-linker-HR1 of SARS virus-linker-HR2 of SARS virus-linker-HR1 of SARS-linker-partial HR2 of SARS.

In specific embodiments, the Five-Helix proteins comprise HR1 components having the sequence of from about residue 900 to about residue 1013 of SEQ ID NO:1, HR2 components having the sequence of residues from about 1148 to about 1193 of SEQ ID NO:1, and amino acid linkers therebetween. The HR1 and/or HR2 SARS sequence can be altered by the addition, deletion or substitution of one or more amino acid residues to produce additional Five-Helix proteins.

In a specific embodiment, a Five-Helix protein comprises three HR1 repeats having the following amino acid sequence: the residues 900-1005 of SEQ ID NO:1, and at least two and up to, but not including, three HR2 repeats having the following amino acid sequence: the residues 1151-1185 of SEQ ID NO:1. In another embodiment, a Five-Helix protein comprises three HR1 repeats having the following amino acid sequence: the residues 900-974 of SEQ ID NO:1, and at least two and up to, but not including, three HR2 repeats having the following sequence: the residues 1148-1193 of SEQ ID NO:1. In another specific embodiment, a Five-Helix protein comprises three HR1 repeats having the amino acid sequence: the residues 900-1005 of SEQ ID NO:1, and at least two and up to, but not including, three HR2 repeats having the following sequence: the residues 1148-1193 of SEQ ID NO:1. In a further specific embodiment, a five-helix protein comprises three HR1 repeats having the amino acid sequence: the residues 900-974 of SEQ ID NO:1, and at least two and up to, but not including, three HR2 repeats having the following amino acid sequence: the residues 1151-1185 of SEQ ID NO:1.

According to an embodiment of the present invention, a Five-Helix protein has the sequence of residues 1-243 of SEQ ID NO:34, whereas the corresponding Six-Helix the sequence of SEQ ID NO:34:

MVLYENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAGGGSSGGSGGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGGGSSGGSGGVLYENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAGGGSSGGSGGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGGGSSGGSGGVLYENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAGGGSSGGSGGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQEL (SEQ ID NO:34).

According to another embodiment of the present invention, a Five-Helix protein has the sequence of residues 1-237 of SEQ ID NO:35, whereas the corresponding Six-Helix the sequence of SEQ ID NO:35:

MTALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSGGGSSGGSGGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGGGSSGGSGGTALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSGGGSSGGSGGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGGGSSGGSGGTALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSGGGSSGGSGGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQEL (SEQ ID NO:35).

According to an alternative embodiment of the present invention, a Five-Helix protein has the sequence of residues 1-237 of SEQ ID NO:36, whereas the corresponding Six-Helix the sequence of SEQ ID NO:36:

MQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSEGGSSGGSGGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGGGSSGGSGGQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSEGGSSGGSGGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGGGSSGGSGGQIDRLITGRLQSL

QTYVTQQLIRAAEIRASANLAATKMSEGGSSGGSSGGDISGINASVVNIQKEIDRLNEVAKNLNESLID
LQEL (SEQ ID NO:36).

According to an alternative embodiment of the present invention, a Five-Helix protein has the sequence of residues 1-317 of SEQ ID NO:37, whereas the corresponding Six-Helix the sequence of SEQ
 5 ID NO:37:

MGIGVTQNVLYENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSGGGSSGGGS
GGLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKGGSSGGSSGGGIGVTQNVLYE
NQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSGGGSSGGSSGGLGDISGINAS
VVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKGGSSGGSSGGGIGVTQNVLYENQKQIANQFNKA
 10 ISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSGGGSSGGSSGGLGDISGINASVVNIQKEIDRLN
EVAKNL NESLIDLQELGKYEQYIK (SEQ ID NO:37).

The amino acid sequence of each of the components (the HR1 repeats and the HR2 repeats) can vary widely, provided that the resulting repeats form a trimer of hairpins. The sequences provided herein can be modified by the addition, deletion, or substitution of one or more amino acid(s). Amino acid
 15 residues in the C-peptide helix (HR2), particularly the amino acid residues that are solvent exposed (have high solvent accessibility) in the trimer of hairpin structure, can be changed to other amino acid residues with considerable flexibility. For example, such amino acid residues can generally be changed to (substituted with) naturally-occurring amino acid residue except, in general, proline, which can be used as a cap. Amino acid residues in the N-peptide helix (HR1) can also be changed. In the N-peptide, the
 20 amino acid residues that are buried in the coiled-coil trimer (buried in the trimer interface) can be substituted with amino acid residues that can be accommodated in the interface. Such characteristics of amino acid as size, charge and hydrophobicity must be taken into consideration in selecting amino acid substitutions in the N-peptide.

As discussed above, the amino acid residue composition of Five-Helix of the present invention
 25 can vary greatly. In those instances in which Five-Helix is used in therapeutic applications, it must present a surface or region that is available for binding or interacting with coronavirus HR2. That is, the remaining surface of Five-Helix must be present in such a manner or conformation that it is available to bind or interact with coronavirus HR2. In the cases in which Five-Helix is used as a drug-screening tool or an antibody-screening tool or as a vaccine or immunogen, Five-Helix need not bind or interact with
 30 coronavirus HR2.

The HR1 components of Five-Helix proteins of the present invention can vary in amino acid composition (type and number). They will generally comprise from about 20 to about 100 amino acid residues of HR1 of coronavirus (e.g., human) S2 protein and each component can comprise any number of amino acid residues from about 20 to about 100. They will generally comprise from about 20 to about

60 amino acid residues of HR2 of coronavirus (e.g., human) S2 protein and each can comprise any number of amino acid residues from about 20 to about 60. The amino acid composition of each HR1 component in a Five-Helix protein can be the same or different. The amino acid composition of each HR2 component in a Five-Helix protein can be the same or different. The amino acid residues included in HR1 and HR2 can occur sequentially/consecutively in the corresponding region of a coronavirus or may be nonconsecutive amino acids (e.g., amino acids that are not consecutive in the coronavirus HR1 and/or HR2 can make up an HR1 or HR2 of a Five-Helix).

In further embodiments, Five-Helix protein can comprise equivalent HR1 and HR2 components from other coronaviruses, which can be identified using known methods, such as Multicoil and Learncoil VMF. See, for example, Wolf, E. et al. Protein Science (June 1997) 6: 1179-1189, describing Multicoil and Singh, M. et al. Journal of Molecular Biology (1999) 290: 1031-1041, describing Learncoil VMF. Alternatively, Five-Helix proteins of the present invention can differ from a sequence presented herein by addition, deletion, substitution or alteration of at least one amino acid residue. The linkers can be of any length or composition, provided that the Five-Helix protein conformation, described herein, is retained. Five-Helix can be an L-amino acid protein, a D-amino acid protein or a combination of L-amino acid residues and D-amino acid residues; these residues can be modified residues or non naturally occurring residues.

7. The Retro-Inverso Analogues

The present invention further relates to the inhibitors that are retro-inverso analogues of the inhibitors derived from HR1 and/or HR2 of the coronaviruses such as SARS-CoV. The *in vivo* stability of a peptide is critical for its application in therapies. The retro-inverso analogues have higher stability than their counterparts, while reserving the capability of binding the pre-hairpin intermediate of S protein and inhibiting coronavirus inhibition.

7.1. The Retro-Inverso Peptides

The retro-inverso analogue of a peptide is more stable than the peptide, while it often maintains the biological activities of the peptide. A retro-inverso peptide is an isomer of a linear peptide in which the direction of the amino acid sequence is reversed, and the chirality of each amino acid residue is inverted (Goodman & Chorev, *Acc. Chem. Res.* 12:1-7 (1979); Chorev & Goodman, *M. Acc. Chem. Res.* 26:266-273 (1993); Chorev & Goodman, *M. Trends Biotechnol.* 13:438-445 (1995); Fletcher & Campbell, *Chem. Rev.* 98:763-795(1998); Taylor, et al., *J. Pharmacol. Exp. Ther.* 295:190-194 (2000)). This results in inversion of each peptide bond within the peptide sequence, while the overall topology of

the side-chains is maintained, except for the terminal groups. Hence, when a retro-inverso peptide is superimposed onto the parent peptide in an antiparallel fashion, the overall topology of the side-chains is maintained (See Fig. 3).

Because most proteins are composed of L-amino acids, retro-inverso peptides are usually peptides composed of D-amino acids. As used herein, D-chirality is indicated with the lowercase, while L-chirality is indicated with the uppercase. For amino acids comprising more than one chiral center, like isoleucine and threonine, the amino acid used in the retro-inverso analog may comprise any combination of the possible configurations of the said amino acid. As used herein, "i" may represent (D)-Isoleucine, (D)-allo-isoleucine, or (L)-allo-isoleucine, while "t" may represent (D) threonine, (D)-allo-threonine, or (L)-allo-threonine.

When the interaction of a peptide ligand with another peptide or protein is mainly dependent on side-chain topology, and much less so on contacts established with the peptide amide backbone, the binding affinity of the parent sequence can be preserved in the retro-inverso analog. There are several examples of retro-inverso peptides, which maintain the same biological activity of the parent peptides (for recent examples see Levi *et al.*, *Res. Human Retroviruses* 16:59-65 (2000); Taylor *et al.*, 2000; Pescarolo *et al.*, *FASEB J.* 15:31-33 (2000); Chen *et al.*, *J. Med. Chem.* 45 1624-1632 (2002); D'Ursi *et al.*, *J. Med. Chem.* 46 1807-1810 (2003)). In contrast, retro-inverso analogs were biologically inactive in a few cases. A possible cause might be the non-complementarity of the end group with the corresponding end groups of the linear peptide, as for (Fletcher & Campbell, *Chem. Rev.* 98:763-795(1998)).

This non-complementarity, however, is unlikely to render inactive the retro-inverso analogues of the heptad repeats of viral fusion proteins. These peptides are thought to act in a dominant-negative manner, by substituting for one or more components of the trimer, which results from collapse of the HR 1 and 2, thereby preventing the conformational changes necessary for fusion (Lambert *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 93:2186-2191 (1996); Chan & Kim, *Cell* 93:681-684 (1998); Sodroski, *Cell* 99:243-246 (1999)). This interaction occurs over a large surface area, and does not depend on a single, specific contact between the interacting peptide pairs. For instance, N-terminal truncations of the potent HIV-1 fusion inhibitor DP-178 give rise to fully active peptides (Lawless *et al.*, *Biochemistry* 35:13697-13708 (1996)).

Furthermore, retro-inverso peptides invariably show extended stability in serum, because the inverted amide backbone makes them poor substrates for serum proteases (Goodman & Chorev, *Acc. Chem. Res.* 12:1-7 (1979); Chorev & Goodman, *M. Acc. Chem. Res.* 26:266-273 (1993); Chorev & Goodman, *M. Trends Biotechnol.* 13:438-445 (1995); Levi *et al.*, *Res. Human Retroviruses* 16:59-65 (2000); Taylor *et al.*, 2000; Pescarolo *et al.*, *FASEB J.* 15:31-33 (2000); Chen *et al.*, *J. Med. Chem.* 45 1624-1632 (2002); D'Ursi *et al.*, *J. Med. Chem.* 46 1807-1810 (2003)). The increased stability in serum

is a key advantage of retro-inverso peptides, since it may result in increased serum half-life and improved PK properties. Indeed, D'Ursi *et al. J. Med. Chem.* 46 1807-1810 (2003)) show that the retro-inverso analog of an octapeptide inhibitor of Feline Immunodeficiency Virus is only 4-fold less active than the parent peptide in vitro, but it is 6-fold more potent in the presence of 50% Normal Cat Serum.

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7.2. The Retro-Inverso Analogues of Coronavirus HR Peptides

According to an embodiment of the present invention, the inhibitor comprises a retro-inverso analogue of any of the inhibitory peptides or proteins disclosed herein, including C-peptides, N-peptides, Five-Helix proteins, Heptad Repeat 2 (HR2) peptides, and Heptad Repeat 1 (HR1) coiled-coil peptides.

10 The inhibitor comprises a retro-inverso analogue of a sequence from coronavirus heptad repeat 1 or heptad repeat 2.

According to an embodiment of the present invention, the coronavirus is a human coronavirus or a mouse coronavirus. According to a preferred embodiment of the present invention, the coronavirus is a human SARS coronavirus (SARS-CoV).

15 According to an embodiment of the present invention, the inhibitor comprises a retro-inverso analogue of a sequence from coronavirus heptad repeat 2 (HR2); the sequence has a length of at least 20 amino acid residues. According to a preferred embodiment of the present invention, the inhibitor is a retro-inverso analogue of HR2 peptide or C-peptide disclosed herein. The inhibitor comprises a retro-inverso analogue of the predicted HR2 region. The HR2 sequence has a length of from about 20 to about
20 60 amino acid residues, or preferably from about 20 to about 50 amino acid residues. Amino acid residues present in these inhibitors may be a retro-inverso sequence of amino acid residues that occur sequentially in a coronavirus HR2 or may be amino acid residues that are separated by other (intervening) amino acid residues in the coronavirus HR2.

25 Like corresponding HR2 inhibitors, the inhibitors can bind to the HR1 regions of the pre-hairpin intermediate in a dominant-negative manner to block formation of the hairpin structures, and thereby prevent the infection of coronavirus to the cells.

Possible inhibitors include, but are not limited to, peptides comprising a retro-inverso analogue of the amino acid sequences disclosed in sections 2-5 and peptides that have an amino acid sequence sufficiently similar to any of those sequences to function in the same manner. In particular embodiments,
30 a inhibitor comprises a retro-inverso analogue of some or all of the amino acid sequence of SARS virus HR2, as disclosed in section 2. The SARS fusion inhibitors comprise from about 20 amino acid residues to about 60 amino acid residues. Amino acid residues present in these retro-inverso inhibitors may be a sequence of amino acid residues that occur consecutively (contiguously) in a coronavirus HR2 or may be

amino acid residues that are separated by other (intervening) amino acid residues noncontiguously) in the coronavirus HR2.

The inhibitor can comprise a retro-inverso analogue of any of the sequences derived from SEQ ID NO:2, as disclosed in section 2. According to an embodiment of the present invention, the inhibitor can be any of the sequences derived from the retro-inverso analogue of SEQ ID NO:2, which is
1wvywpwkiyqeykgleqldilsenlnkavenlrldiekqinvvsanigsidgldvdp. Examples of such HR2 retro-inverso analogues are partial or full sequence of the retro-inverso analogue of SEQ ID NO:2. HR2 retro-inverso analogues can consist of amino acid residues from N to (N+M-1) of the analogue, wherein M is the length of the HR2 peptide, and N can be any integral number from 1 to (58-M).

According to a further preferred embodiment of the present invention, the inhibitor comprises
1ldilsenlnkavenlrldiekqinvvsanigsidgl, which is the retro-inverso analogue of SEQ ID NO:3.

According to an embodiment of the present invention, the fusion inhibitor of a coronavirus comprises a retro-inverso analogue of a sequence of at least 28 amino acid residues from coronavirus heptad repeat 1 (HR1). Like corresponding HR2 inhibitors, the retro-inverso inhibitors can bind to the HR1 regions of the pre-hairpin intermediate in a dominant-negative manner to block formation of the hairpin structures, and thereby prevent the infection of coronavirus to the cells. The inhibitor is retro-inverso HR1 peptide or N-peptide. The inhibitor can be the retro-inverso analogue of any of the HR1 or N-peptide inhibitors disclosed herein.

According to an alternative embodiment of the present invention, the inhibitor comprises a retro-inverso analogue of soluble HR1-IQ peptides or HR1-IZ peptides, which are comprised of a soluble trimeric coiled coil fused to an HR1 peptide, which has the sequence of at least a portion of HR1 of coronavirus S2 protein. The peptides comprise a peptide of at least 15 amino acid residues from HR1 of a coronavirus fused in the proper heptad repeat register to a soluble trimeric coiled coil, such as GCN4-pIqI or IZ. The HR1 region component of these peptides can be of varying length and will typically be from about 15 to about 50 amino acid residues from the coronavirus HR1 region.

The inhibitor can be the retro-inverso analogue of any of the HR1 coiled-coil inhibitors disclosed herein.

According to a further preferred embodiment of the present invention, the inhibitor comprises a peptide having a retro-inverso analogue of sequence selected from the group consisting of the partial or full sequence of SEQ ID NO: 16-28.

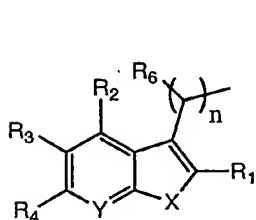
8. Inhibitors Containing Tryptophan Analogs

The present invention further relates to C-peptide and N-peptide coronavirus inhibitors containing one or more tryptophan analogs.

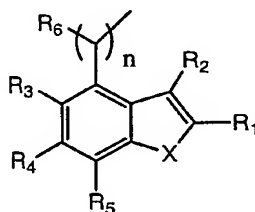
A variety of different C-peptide and N-peptide inhibitors can be obtained based on the disclosure provided herein including the identification of HR1 and HR2 sequences, and the use of tryptophan analogs. Tryptophan analogs can be used to increase serum half-life and improve pharmacokinetic properties. Additional regions that may be present include additional coronavirus regions and other types of regions such as enhancer regions. Enhancer regions either substantially maintain or enhance either coronavirus inhibitor properties or pharmacokinetic properties. Examples of enhancer regions are provided in Barney *et al.*, International Publication Number WO 01/03723, published January 18, 2001.

8.1. Tryptophan Analogs

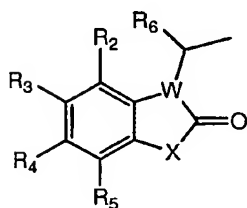
Tryptophan analogs can be present in either D or L stereochemistry. Preferred tryptophan analogs are modified amino acids having an R group (D or L stereochemistry) independently selected from the group consisting of:



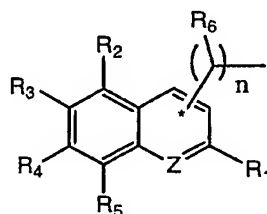
I



II



III



IV

wherein X is O, S, or NR₇;

Y is C-R₅ or N;

W is N or CH;

Z is CH or N;

5 R₁, R₂, and R₅ are independently selected from the group consisting of hydrogen, halogen, C₁-C₆ alkyl, C₁-C₆ alkylamino, amino, and carboxyl;

R₃ and R₄ are either (1) independently selected from the group consisting of hydrogen, halogen, C₁-C₆ alkyl, C₁-C₆ alkylamino, amino, and carboxyl; or (2) joined together to provide a fused benzene ring;

10 R₆ is H or methyl;

R₇ is H or linear, branched or cyclic C₁-C₆ alkyl; and

n is 0 or 1;

further provided that in the case of formula I, R₁ may also be CH₂ where there is a bond from this CH₂ to the alpha-NH of the modified amino acid.

15 Specific examples of different tryptophan analogs are shown in Figures 4A, 4B and 4C. The tryptophan analogs are shown as amino acids. Amino acids are joined together in a peptide by peptide bonds.

8.2. Additional HR Peptide Structures

20 Preferably, enhancer regions are shorter length amino acid sequences up to about eight amino acids. In an embodiment of the present invention, the enhancer region at the N-terminus is XQEXEQK (SEQ ID NO:38), whereas the enhancer region at the C-terminus is XPXYVXL (SEQ ID NO:39), where each X is independently either tryptophan or a tryptophan analog.

25 According to an embodiment of the present invention, tryptophan analogs may be present in the HR2 based region or an additional amino acid region located at the N-terminus or C-terminus of the HR2 based region.

In an embodiment of the present invention the C-peptide consists of:

(a) a HR2 based region comprising about 18 amino acids of SEQ ID NO: 2 optionally modified with one or more conservative substitutions thereof;

30 (b) an optionally present N-terminus enhancer group joined to the N-terminus of the HR2 based region;

(c) an optionally present C-terminus enhancer group joined to the C-terminus of the HR2 based region;

provided that at least one tryptophan analog is present,

further provided that the C-peptide N-terminus, C-terminus, or both the N-terminus and C-terminus may contain a protecting group.

In a more preferred embodiment a C-peptide consists of

(a) a HR2 based region consisting of a sequence selected from the group consisting of:

5 amino acid residues from N to (N+M-1) of SEQ ID NO:2, wherein M is the length of the HR2 peptide, and N can be any integral number from 1 to (58-M).

(b) an optionally present N-terminus enhancer group, preferably WQEWEQKI (SEQ ID NO:40), where one or both tryptophans may be replaced with a tryptophan analog;

10 (c) an optionally present C-terminus enhancer group, preferably, WPWYVWL (SEQ ID NO:41) where one, two or all three tryptophans may be replaced with a tryptophan analog;

provided that at least one tryptophan analog is present; and

provided that the C-peptide N-terminus, C-terminus, or both the N-terminus and C-terminus may contain a protecting group.

15 In a further preferred embodiment, According to a preferred embodiment of the present invention, the HR2 peptide has a sequence of Z-LGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLXPXYVXL (SEQ ID NO:42), and

a variant thereof comprising one or more conservative substitutions;

wherein Z is an optionally present enhancer group, wherein each X is independently either tryptophan or a tryptophan analog;

20 provided that the C-peptide N-terminus, C-terminus, or both the N-terminus and C-terminus may contain a protecting group.

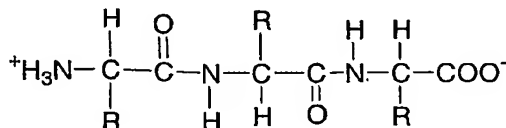
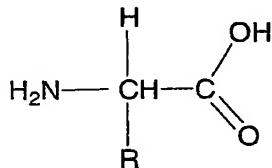
9. Inhibitors Containing Lactam Bridges

25 The introduction of one or more lactam bridges into a C-peptide or N-peptide can be used to stabilize a conformation providing advantages over the corresponding structure not containing lactam bridges. Advantages that can be provided include one or more of the following; resistance to proteolytic enzyme, improved serum half-life, enhanced biological activity, and enhanced pharmacokinetic properties. The same enhanced stability, coupled with cross-reactivity of the antibody response between the linear and the corresponding cyclic constrained sequences, may make the peptides more suitable than
30 their linear counterparts for generation of an immune response as part of a vaccine.

9.1. Lactam Bridges

A lactam bridge is formed between a carboxyl moiety of a first amino acid and an amino moiety of a second amino acid. The carboxyl and amino moieties form an amide bond.

The general structure of an individual amino acid and amino acids in a peptide (tripeptide with ionized amino and carboxyl groups) are as follows (where R is an amino acid R group):



Introduction of lactam bridges into a particular peptide can make use of amino acids having suitable groups or involve modifying the peptide to insert one or more suitable amino acids. Suitable amino acids for forming a lactam bridge include naturally occurring amino acid and non-naturally occurring amino acids.

Suitable amino acids are amino acid pairs where one amino acid contains a free amino and another contains a free carboxyl. Examples of amino acids containing an R-group carboxyl moiety include naturally occurring amino acids such as aspartic acid and glutamic acid, and non-naturally occurring amino acids such as 2-aminohexanedioic acid and 2-aminoheptanedioic acid. Examples of amino acids containing an R-group amino moiety include naturally occurring amino acids such as lysine and ornithine, and non-naturally occurring amino acids such as diaminobutyric acid and diaminopropanoic acid.

Cyclization in an (i, i + 3), (i, i + 4) and (i, i + 7) spaced side chain to a side chain lactam bridge stabilizes turn conformations and helical segments. (Taylor *Biopolymers (Peptide Science)* 66:49-75, 2002.) A peptide can be modified to contain amino acids with appropriate groups (carboxyl and amino pairs) suitably spaced apart.

9.2. The Structures of HR Peptides Containing Lactam Bridges

In an embodiment of the present invention, the C-peptide consists of a sequence selected from the group consisting of: any of the sequences derived from SEQ ID NO:2, as disclosed in section 2;

that is (a) modified to contain one or more lactam bridges each of which is independently in an (i, i + 3), (i, i + 4) or (i, i + 7) orientation; (b) optionally modified to contain one or more conservative substitutions; (c) optionally modified to contain a C-terminus deletion; and (d) optionally modified to contain a N-terminus deletion,

5 provided that the N-terminus, C-terminus, or both the N-terminus and C-terminus may contain a protecting group.

Examples of such HR2 peptides are partial or full sequence of SEQ ID NO:2. HR2 retro-inverso analogues can consist of amino acid residues from N to (N+M-1) of SEQ ID NO:33, wherein M is the length of the HR2 peptide, and N can be any integral number from 1 to (58-M).

10 Examples of peptides containing (i, 1+3), (i, i+4), or (i, i+7) lactam bridges are:
(i,i+3)

1. (Lys) (Asp)
P(Asp)VD(Lys)GDISGINASVVNIQKEIDRLNEVAKNLNES
└────────┘
(SEQ ID NO:43)

(i,i+4)
2. (Dab) (Glu)
DVDLG(Glu)ISG(Dab)NASVVNIQKEIDRLNEVAKNLNESL
└────────┘
(SEQ ID NO:44)

(i,i+7)
3. (Glu) (Orn)
VDLGDISGINA(Orn)VVNIQK(Glu)IDRLNEVAKNLNESLI
└────────┘
(SEQ ID NO:45)

15 10. The Production of the Inhibitors

The inhibitors can be produced by a variety of methods, including by means of recombinant DNA or RNA methods and / or known chemical synthetic methods. They can also be obtained from a library of peptides, such as a combinatorial peptide library. They may be capped or uncapped with certain amino

acids. Recombinant versions can contain helix capping residues on the N- terminus, the C-terminus or both. Possible capping residues for the N-terminus are amino acid T, D and S and for the C-terminus include amino acid G, R, H, N, and K.

The peptides may also contain a C-terminus cap (also referred to herein as a C-terminus “protecting group”) or a N-terminus cap (also referred to herein as a N-terminus “protecting group”). A C-terminus protecting group is a modification to the C-terminal amino acid that reduces the reactivity of the carboxyl terminus under *in vivo* conditions. The carboxyl terminus is preferably modified at the α -carbonyl group. Examples of modification include chemical groups such as amide, methylamide, and ethylamide.

An N-terminus protecting group is a modification to the N-terminal amino acid that reduces the reactivity of the amino terminus under *in vivo* conditions. The N-terminal amino acid can be modified with groups such as acetyl, propionyl, succinyl, benzyl, benzyloxycarbonyl or *t*-butyloxycarbonyl.

10.1. The chemical synthesis of the peptides

According to an embodiment of the present invention, the peptides are produced using standard techniques including those involving step-wise chemical synthesis. Techniques for chemical synthesis of peptides are well known in the art. (See *e.g.*, Vincent, in *Peptide and Protein Drug Delivery*, New York, N.Y., Dekker, 1990; E. Atherton & R. C. Sheppard, *Solid Phase Peptide Synthesis. A Practical Approach*. IRL Press, Oxford, England, 1989; J. Jones, *The Chemical Synthesis of Peptides*, Oxford University Press, New York, 1991; M. Bodanszky & A. Bodanszky, *The Practice of Peptide Synthesis*, 2nd ed., Springer-Verlag, Berlin Heidelberg, 1994; Fields, G., Ed., *Solid-Phase Peptide Synthesis*. Methods Enzymol. 289, Academic press, New York, 1997).

Tryptophan analogs can be produced and incorporated into a peptide taking into account well known techniques. Different tryptophan analogs can be obtained from commercial sources such as RSP Amino Acid Analogues Inc., Bachem, and Sigma; or based on techniques such as those described by Huber *et al.*, *Tetrahedron Letters* 43:6729-6733, 2002, Fredrickson *et al.*, *European Journal of Pharmacology* 458(1-2):111-118, 2003, Mohammadi *et al.*, *Biochemistry* 40(34):10273-10283, 2001, Rajh *et al.*, *International Journal of Peptide & Protein Research* 14(1):68-79, 1979, and Cady *et al.*, *Archives of Biochemistry and Biophysics* 291(2):326-333, 1991.

Examples of techniques that can be used to produce and study peptides containing lactam bridges are provided by Taylor, *Biopolymers (Peptide Science)* 66:49-75, 2002 and Judice *et al.*, *Proc. Natl. Acad. Sci. USA* 94:13426-13430, 1997.

The retro-inverso peptides of the present invention may be chemically synthesized, using the same methods utilized for the synthesis of normal peptides. See, *e.g.*, *J. Biol. Chem.* 270:11921-11926

(1995), Briand et al., *Proc. Natl. Acad. Sci. U.S.A.* 94:12545-12550 (1997), Fletcher & Campbell, *Chem. Rev.* 98:763-795(1998), Levi et al., *Res. Human Retroviruses* 16:59-65 (2000); Taylor et al., 2000; Pescarolo et al., *FASEB J.* 15:31-33 (2000); Chen et al., *J. Med. Chem.* 45 1624-1632 (2002); D'Ursi et al., *J. Med. Chem.* 46 1807-1810 (2003)

5

10.2 The production of Five-Helix proteins with recombinant DNA technologies

The present invention further relates to DNA encoding Five-Helix; methods of producing Five-Helix. Five-Helix protein can be produced by a variety of methods. For example, it can be produced from a larger protein, such as 6-Helix, by enzymatic (trypsin) digestion. Alternatively, it can be produced, using known methods and expression systems, by expressing Five-Helix protein-encoding DNA, which can be a single DNA that encodes the entire Five-Helix protein or two or more DNA "units", each of which encodes a portion (e.g., one or more HR1 component, one or more HR2 components) of Five-Helix protein.

The yield of expression and purification of Five-Helix can be significantly improved by direct expression of the Five-Helix gene in an appropriate host cell, such as *E. coli*. In this approach, the Five-Helix gene encodes the residues present in the final Five-Helix protein. A C-terminal His-tag can be attached to facilitate purification (with or without a protease cleavage site to later remove the tag). The protein can then be used directly without the proteolytic cleavage and unfolding steps required for producing Five-Helix starting from Six-Helix.

This Five-Helix molecule may be expressed as a folded active molecule, allowing its use in biological selections or screens for optimizing its properties. Alternatively, protein synthetic methods can be used to produce Five-Helix protein. The HR1 and HR2 components of Five-Helix can be joined covalently (such as by means of a linker of at least one (one or more) amino acid residues) or by other means which results in formation of a protein which is stable under physiological conditions and is correctly folded such that the remaining surface of Five Helix is presented so that it is available to bind or interact with coronavirus HR2. In the embodiments in which there are three HR1 components and more than two (but less than three complete) HR2 components, the components can be similarly joined.

Five-Helix can be assessed to determine if it acts on the virus before it enters the cell. Five-Helix should be soluble under physiological conditions and should also be stable under physiological conditions.

It should also be possible to generate 5-Helix variants with an increased molecular weight (by oligomerization or tethering to a large protein) to reduce the rate of kidney clearance. In addition, Five-Helix dimers can be made by disulfide crosslinking, to produce a molecule filtered to a lesser extent than the Five-Helix "monomer". Thus, it is reasonable to expect that dimers might have an enhanced

bioavailability when compared to that of the C-peptides. Bioavailability of Five-Helix can be affected (enhanced) by producing variants in nonbacterial cells, in which the protein would be glycosylated as it is produced. Alternatively, glycosylation sites can be engineered into Five-Helix.

Another embodiment of this invention is a complex of Five-Helix tethered (with an appropriate linker, which can be made of amino acid residues, or can be a chemical or synthetic linker) to a component, such as a ligand (protein or small molecule) that binds coronavirus envelope protein. The complex is useful as a therapeutic agent, because the ligand will increase the effective concentration of the tethered Five-Helix moiety in the vicinity of the coronavirus envelope protein.

11. Utilities of the Coronavirus Inhibitors

The present invention relates to methods in which the peptides and Five-Helix proteins are used. The peptides and Five-Helix are useful as therapeutic agents or drugs to prevent coronavirus infection or reduce the extent to which it occurs; as a reagent for identifying (screening for) or designing other therapeutics or prophylactics effective against coronavirus infection.

In one embodiment of the present invention, the peptides and Five-Helix are used to reduce coronavirus infection in an individual. In this embodiment, the peptides or Five-Helix is administered to an individual in sufficient quantity to reduce (totally or partially) infection of the individual's cells. That is, a dose of the peptides or Five-Helix sufficient to reduce coronavirus infection (an effective dose) is administered in such a manner (e.g., by injection, topical administration, intranasal route, intravenous route) that it inhibits (totally or partially) coronavirus entry into cells. The peptides or Five-Helix can be administered to an individual who is infected, to reduce further infection, or to an uninfected individual to prevent infection or reduce the extent to which infection occurs. The Five-Helix can also be administered via expression of Five-Helix-encoding DNA in appropriate host cells or vectors.

The present invention provides a method of inhibiting infection of human cells by coronavirus in an individual, comprising administering to the individual a pharmaceutical composition comprising the retro-inverso analogue of a SARS-CoV HR peptide in sufficient quantity to inhibit infection of human cells by the SARS virus, and a pharmaceutical acceptable carrier, wherein infection of human cells is inhibited.

11.1 Administration

HR-peptide coronavirus inhibitors can be formulated and administered to a subject using the guidance provided herein along with techniques well known in the art. The preferred route of administration ensures that an effective amount of compound reaches the target. Guidelines for

pharmaceutical administration in general are provided in, for example, *Remington's Pharmaceutical Sciences 18th Edition*, Ed. Gennaro, Mack Publishing, 1990, and *Modern Pharmaceutics 2nd Edition*, Eds. Banker and Rhodes, Marcel Dekker, Inc., 1990.

HR-peptide coronavirus inhibitors can be prepared as acidic or basic salts. Pharmaceutically acceptable salts (in the form of water- or oil-soluble or dispersible products) include conventional non-toxic salts or the quaternary ammonium salts that are formed, *e.g.*, from inorganic or organic acids or bases. Examples of such salts include acid addition salts such as acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, and undecanoate; and base salts such as ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine and lysine.

HR-peptide coronavirus inhibitors can be administered using different routes such as an intravenous route, an intraperitoneal route, a subcutaneous route, a topical route, an intramuscular route, or an intranasal route. When administered by injection, the injectable solution or suspension may be formulated using suitable non-toxic, parenterally-acceptable diluents or solvents, such as Ringer's solution or isotonic sodium chloride solution, or suitable dispersing or wetting and suspending agents, such as sterile, bland, fixed oils, including synthetic mono- or diglycerides, and fatty acids, including oleic acid.

Pharmaceutically acceptable carriers facilitate storage and administration of a HR-peptide to a patient. Pharmaceutically acceptable carriers may contain different components such as a buffer, sterile water for injection, normal saline or phosphate buffered saline, sucrose, histidine, salts and polysorbate.

Suitable dosing regimens are preferably determined taking into account factors well known in the art including type of subject being dosed; age, weight, sex and medical condition of the subject; the route of administration; the renal and hepatic function of the subject; the desired effect; and the particular compound employed.

Optimal precision in achieving concentrations of drug within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a drug. The daily dose for a subject is expected to be between 0.01 and 1,000 mg per subject per day.

HR-peptide coronavirus inhibitors can be provided in kit. Such a kit typically contains an active compound in dosage forms for administration. A dosage form contains a sufficient amount of active compound such that a beneficial effect can be obtained when administered to a patient during regular intervals, such as 1 to 6 times a day, during the course of 1 or more days. Preferably, a kit contains instructions indicating the use of the dosage form for treating coronavirus and the amount of dosage form to be taken over a specified time period.

11.2. Immunogens

HR-peptides providing appropriate epitopes may be used as an immunogen for anti-coronavirus antibody production. The immunogen can be used, for example, as a vaccine component or to produce monoclonal or polyclonal antibodies that are subsequently isolated and used to target the coronavirus Spike protein. In an embodiment, the invention relates to a method of eliciting an immune response to coronavirus in an individual, comprising introducing, by an appropriate route, a composition comprising Five-Helix and a physiologically acceptable carrier, in a dose sufficient to elicit the immune response in the individual. Vaccines comprising the peptides or Five-Helix (or a variant or portion thereof) in a physiologically acceptable carrier are the subject of this invention.

Immunogens can be formulated as a vaccine or used for antibody production and isolation taking into account the guidance provided herein along with techniques well known in the art. Techniques for producing and using antibodies are well known in the art. Examples of such techniques are described in Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998, Harlow *et al.*, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988, and Kohler *et al.*, *Nature* 256:495-497, 1975.

Guidelines for pharmaceutical vaccine administration in general are provided in, for example, *Vaccines* Eds. Plotkin and Orenstein, W.B. Sanders Company, 1999; *Remington's Pharmaceutical Sciences 20th Edition*, Ed. Gennaro, Mack Publishing, 2000; and *Modern Pharmaceutics 2nd Edition*, Eds. Banker and Rhodes, Marcel Dekker, Inc., 1990.

A vaccine can be administered in conjunction with an adjuvant. Adjuvants are substances that can assist an immunogen in producing an immune response. Adjuvants can function by different mechanisms such as one or more of the following: increasing the antigen biologic or immunologic half-life; improving antigen delivery to antigen-presenting cells; improving antigen processing and presentation by antigen-presenting cells; and inducing production of immunomodulatory cytokines. (Vogel, *Clinical Infectious Diseases* 30 (suppl. 3):S266-270, 2000.)

A variety of different types of adjuvants can be employed to assist in the production of an immune response. Examples of particular adjuvants include aluminum hydroxide, aluminum phosphate, other salts of aluminum, calcium phosphate, DNA CpG motifs, monophosphoryl lipid A, cholera toxin, *E.*

coli heat-labile toxin, pertussis toxin, muramyl dipeptide, Freund's incomplete adjuvant, MF59, SAF, immunostimulatory complexes, liposomes, biodegradable microspheres, saponins, nonionic block copolymers, muramyl peptide analogues, polyphosphazene, synthetic polynucleotides, IFN- γ , IL-2 and IL-12. (Vogel *Clinical Infectious Diseases* 30(suppl 3):S266-270, 2000, Klein *et al.*, *Journal of Pharmaceutical Sciences* 89, 311-321, 2000.)

Vaccines can be administered by different routes such as subcutaneous, intramuscular, or mucosal. Subcutaneous and intramuscular administration can be performed using, for example, needles or jet-injectors.

Suitable dosing regimens are preferably determined taking into account factors well known in the art including age, weight, sex and medical condition of the patient; the route of administration; the desired effect; and the particular compound employed. The immunogen can be used in multi-dose vaccine formats. It is expected that a dose would consist of the range of 1 μ g to 1.0 mg total polypeptide, in an embodiment of the present invention the range is 0.1 mg to 1.0 mg.

The timing of doses depends upon factors well known in the art. After the initial administration one or more booster doses may subsequently be administered to maintain or boost antibody titers. An example of a dosing regime would be day 1, 1 month, a third dose at either 4, 6 or 12 months, and additional booster doses at distant times as needed.

The retro-inverso peptides can also be used as vaccines against SARS. Several studies have documented that the retro-inverso analogs of linear peptide epitopes can be useful as vaccines (Guichard *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 91:9765-9769 (1994); Benkirane *et al.*, *J. Biol. Chem.* 270:11921-11926 (1995); Briand *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 94:12545-12550 (1997); Meziere *et al.*, 1997; Muller & Briand, *Res. Immunol.* 149:55-57 (1998)). More specifically it was shown for Foot-and-Mouse disease virus (FMDV) that (i) antibodies raised against the natural epitope recognize the retro-inverso peptide; (ii) the retro-inverso peptide is able to generate in rabbits an immune response of much higher titer, and longer duration, than the corresponding linear peptide; (iii) the antibodies generated by immunization with the retro-inverso peptide are cross reactive with FMDV viral particles, and (iv) the immune response generated in guinea pigs by immunization with the retro-inverso peptide is protective against FMDV challenge (Briand *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 94:12545-12550 (1997)).

Therefore retro-inverso peptides, corresponding to the sequence of the Heptad Repeats 1 and 2 of the SARS coronavirus, can be used, either as such or, more desirably, conjugated to a protein carrier like OMPC, for the induction of a protective immune response against the virus.

11.3. Other Utilities

In a specific embodiment, the invention relates to a method of identifying a compound or molecule that binds the peptides or Five-Helix and inhibits coronavirus infection of mammalian cells. The compound or molecule to be assessed is referred to as a candidate inhibitor. The method comprises combining a candidate inhibitor and the peptides or Five-Helix under appropriate binding conditions, and determining and selecting the compound or molecule that binds the peptides or Five-Helix. The method optionally further comprises determining and selecting the selected compound or molecule that inhibits coronavirus infection of mammalian (e.g., human) cells, such as in a cell-based assay. Such a compound or molecule may be used to inhibit (totally or partially) coronavirus infection of human or non-human animals.

Other embodiments are within the following claims. While several embodiments have been shown and described, various modifications may be made without departing from the spirit and scope of the present invention.

EXAMPLES

Examples are provided below to further illustrate different features of the present invention. The examples also illustrate useful methodology for practicing the invention. These examples do not limit the claimed invention.

Example 1: The Assays Used to Test the Peptide Activity

The identification of SARS coronavirus HR1 and HR2 sequences provides substrates that can be employed to evaluate the ability of compound to inhibit HR1 and HR2 interactions. Compounds that can be evaluated include C-peptides and N-peptides.

Different types of assay formats can be employed to measure the ability of a compound to inhibit HR1 and HR2 interactions. Detectable labels on either or both HR1 and HR2 can be used to help measure HR1 and HR2 interactions. Examples of different types of labels include fluorescent labels, radioactive labels, and donor-acceptor pairs (e.g., FRET labels).

Peptide activity can also be measured by providing a peptide to a cell infected with the coronavirus. Using techniques well known in the art, different types of assays and endpoints can be measured to evaluate peptide activity. For example, an assay can be performed to measure a compound's ability to prevent virus infection and cell-to-cell spread over a 72-hour incubation; where antiviral activity is assayed by measuring retention of the cells' ability to phagocytose neutral red. An example of such an assay (based on USAMRIID assay) is as follows:

1) Incubate cells with different concentrations of test peptide (may be done in triplicate). The different concentrations may be serial dilutions and should include a control with no peptide. Peptide dilutions can be performed in a suitable buffer (*e.g.*, EMEM, 1% fetal bovine serum, 1.0% L-glutamine (100 X), 1.0% HEPES, 0.1% Funigzone (250 µg/ 20 ml), 0.1% Gentamicin (50 mg/ml)) added to a 96-well microtiter plate of confluent Vero 76 cells (VERO 76, ATCC number CRL-1587). At different drug concentrations wells are inoculated with SARS associated coronavirus (*e.g.*, MOI of 0.01) or are not infected.

2) Plates are incubated at 37°C, 5% CO₂ and examined daily until a desired cytopathic effect is seen in untreated cells (*e.g.*, 4 +CPE). Cell viability can be assessed by measuring neutral red uptake. For example, neutral red can be added to give a final concentration of 0.22 mg/ml and the cells are incubated at 37°C for 1.5 hours. The medium is then removed; wells are rinsed three times with phosphate buffered saline and fixed in 10% buffered formalin for 15 minutes. Retained neutral red is solubilized with 100 µl of a 50% ethanol, 50% 0.01 M NH₄H₂PO₄ (pH 3.5). Plates can then be rocked for 30 minutes at 150 RPM to liberate neutral red and the optical density (at 450 nm) can be measured with a plate reading spectrometer.

Other embodiments are within the following claims. While several embodiments have been shown and described, various modifications may be made without departing from the spirit and scope of the present invention.

Example 2: An assay based on the pseudotyping of HIV cores with SARS-CoV Spike protein.

The present invention also provides a SARS-spike dependent entry assay useful for screening inhibitors of SARS-CoV entry and fusion. In this assay, cells transiently or stably expressing angiotensin converting enzyme-2 (ACE-2) are infected with HIV-1 Luciferase reporter viruses pseudotyped with SARS-CoV Spike protein.

HIV DNA that has a deletion in its envelope sequence and containing firefly luciferase in place of the Nef gene is co-transfected with a mammalian expression vector encoding codon optimized SARS-CoV Spike protein. A plasmid DNA encoding the NL4-3 viral clone, which contains a deletion in its envelope sequence and containing firefly luciferase in place of the Nef gene is co-transfected in 293T cells (a human embryonic kidney cell line stably expressing the Simian Virus 40 Large T antigen) with a mammalian expression vector encoding a codon optimized SARS-CoV Spike protein (See, *e.g.*, Simmons, G., et al., *PNAS* 101:4240-5 (2004)).

The transfected cells produce in their supernatants HIV cores that are pseudotyped with SARS Spike protein. These reporter viruses are capable of entering 293T cells transiently or stably transfected

with expression vectors encoding the angiotensin converting enzyme-2 (ACE-2), a cellular receptor for SARS-CoV (Li, W., et al., *Nature* 426:450-4 (2003); Wong, S.K., et al., *Journal of Biological Chemistry* 279:3197-201 (2004)).

- 5 Infection is monitored by measuring Firefly luciferase activity in cell lysates at 48-72h post-infection. The system offers a dynamic range (30-100 fold above infection with particles lacking virus envelopes). Entry through the ACE-2 receptor has been validated in both in transiently and stably transfected cells by demonstrating that particle entry is blocked in a dose-dependent fashion with antibodies directed to ACE-2.

WHAT IS CLAIMED:

What is claimed is:

1. A coronavirus inhibitor which is selected from the group consisting of
 - a) a Five-Helix protein,
 - 5 b) an HR2 peptide,
 - c) an HR1 peptide,
 - d) an HR1 chimeric peptide,
 - e) a D-peptide which is the retro-inverso analogue of b), c), or d); and
 - f) a modified version of b), c), or d), containing one or more modifications selected from the
- 10 group consisting of i) one or more tryptophan analogs, ii) one or more lactam bridges, and iii) one or more protecting groups.
2. The inhibitor of claim 1 which is derived from S protein of human SARS-CoV.
3. The inhibitor of claim 2 further comprising a capping residue on the N-terminus, the C-terminus or both.
- 15 4. The inhibitor of claim 3 wherein the capping residue on the N-terminus is selected from the group consisting of T, D and S and the capping residue on the C-terminus is selected from the group consisting of G, R, H, N, and K.
5. The inhibitor of claim 2 further comprising an amino-terminal cap.
6. The inhibitor of claim 5 wherein the amino-terminal cap is acetyl or succinimide.
- 20 7. A method of identifying a drug candidate that inhibits coronavirus infection, comprising combining a compound and the inhibitor of claim 1, under conditions appropriate for binding of the compound and the inhibitor to occur, and determining if binding occurs.
8. The method of claim 7 further comprising determining if the compound that binds the inhibitor inhibits coronavirus infection of mammalian cells in a cell-based assay.
- 25 9. A method of inhibiting coronavirus infection of cells in an individual, comprising administering to the individual the inhibitor of claim 1 in sufficient quantity and by an appropriate route, whereby infection of cells in the individual is inhibited.
10. The inhibitor of claim 1 which is a Five-Helix protein being soluble under physiological conditions and comprising three heptad repeat 1 components of coronavirus S2 protein and at least two,

but not three complete, heptad repeat 2 components of coronavirus S2 protein, wherein the components are separated by linkers.

11. The inhibitor of claim 1 wherein the Five-Helix protein has an amino acid sequence selected from the group consisting of:

- 5 the sequence of residues 1-243 of SEQ ID NO:34,
- the sequence of residues 1-237 of SEQ ID NO:35,
- the sequence of residues 1-237 of SEQ ID NO:36, and
- the sequence of residues 1-317 of SEQ ID NO:37.

12. The inhibitor of claim 1 which is an HR2 peptide comprising at least 20 amino acid residues from coronavirus HR2 and is an inhibitor of coronavirus infection of eukaryotic cells.

13. The inhibitor of claim 12 wherein the HR2 peptide has a sequence selected from the group consisting of: amino acid residues 5-50, 9-41, 21-57, 5-41, 30-57, and 1-57 of SEQ ID NO:2, and 1148-1182 and 1148-1185 of SEQ ID NO:1.

14. The inhibitor of claim 1 which is an HR1 chimeric peptide having an amino acid sequence selected from the group consisting of:

- a peptide consisting of SEQ ID NO:16, or residues 2-63 of SEQ ID NO:16;
- a peptide consisting of SEQ ID NO:17, or residues 2-67 of SEQ ID NO:17;
- a peptide consisting of SEQ ID NO:18;
- a peptide consisting of SEQ ID NO:19;
- 20 a peptide consisting of SEQ ID NO:20;
- a peptide consisting of SEQ ID NO:21, or residues 2-56 of SEQ ID NO:20;
- a peptide consisting of SEQ ID NO:22, or residues 2-49 of SEQ ID NO:21;
- a peptide consisting of SEQ ID NO:23, or residues 2-53 of SEQ ID NO:22;
- a peptide consisting of SEQ ID NO:24, or residues 2-67 of SEQ ID NO:23;
- 25 a peptide consisting of SEQ ID NO:25, or residues 2-63 of SEQ ID NO:24;
- a peptide consisting of SEQ ID NO:26;
- a peptide consisting of SEQ ID NO:27;
- a peptide consisting of SEQ ID NO:28, or residues 1-49 of SEQ ID NO:25;
- a peptide consisting of SEQ ID NO:29, or residues 1-49 of SEQ ID NO:26;
- 30 a peptide consisting of SEQ ID NO:30;
- a peptide consisting of SEQ ID NO:31; and
- a peptide consisting of SEQ ID NO:32.

15. The inhibitor of claim 1 which is an HR1 peptide comprising about 28 amino acid residues from HR1 of a coronavirus.

16. The inhibitor of claim 15 wherein the HR1 peptide have an amino acid sequence comprising amino acid residues from about amino acid residue 900 to amino acid residue 974 of the sequence of SEQ ID NO:1 or amino acid residues from about 889 to about 1005 of the sequence of SEQ ID NO:1.

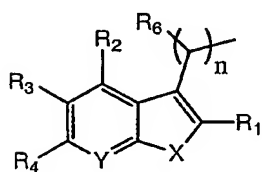
17. The inhibitor of claim 16 wherein the amino acid sequence is selected from the group consisting of sequences of residues 896-972, 900-938, 914-949, 922-956, 943-980, and 943-981 of SEQ ID NO:1.

18. The inhibitor of claim 1 which is the retro-inverso analogue of an HR2 peptide, an HR1 peptide, or an HR1 chimeric peptide.

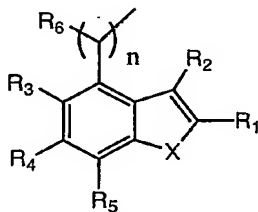
19. The inhibitor of claim 1 comprising,
an HR region selected from the HR1 or HR2 sequence of coronavirus, optionally modified with one or more conservative substitutions, and
one or more tryptophan analogs,

wherein said one or more tryptophan analogs may be present in said HR based region or an additional amino acid region located at the N-terminus or C-terminus of said HR based region.

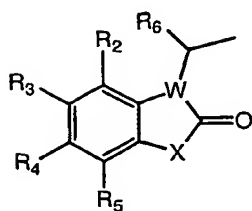
20. The peptide of claim 19, wherein each tryptophan analog is a modified amino acid having an R group independently selected from the group consisting of:



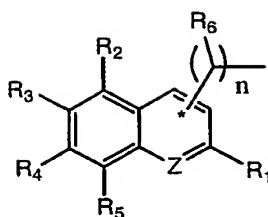
I



II



III



IV

wherein X is O, S, or NR₇;

Y is C-R₅ or N;

5 W is N or CH;

Z is CH or N;

R₁, R₂, and R₅ are independently selected from the group consisting of hydrogen, halogen, C₁-C₆ alkyl, C₁-C₆ alkylamino, amino, and carboxyl;

10 R₃ and R₄ are either (1) independently selected from the group consisting of hydrogen, halogen, C₁-C₆ alkyl, C₁-C₆ alkylamino, amino, and carboxyl; or (2) joined together to provide a fused benzene ring;

R₆ is H or methyl;

R₇ is H or linear, branched or cyclic C₁-C₆ alkyl; and

n is 0 or 1;

15 further provided that in the case of formula I, R₁ may also be CH₂ where there is a bond from this CH₂ to the alpha-NH of said modified amino acid.

21. The peptide of claim 20, wherein said peptide consists of:

said HR region,

an optionally present N-terminus enhancer group joined to the N-terminus of said HR region, and

20 an optionally present C-terminus enhancer group joined to the C-terminus of said HR region,

provided that at least one tryptophan analog is present,

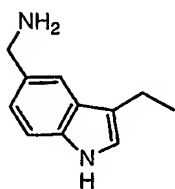
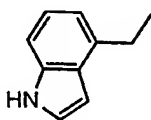
further provided that said N-terminus, said C-terminus, or both said N-terminus and C-terminus of said peptide may contain a protecting group.

22. The peptide of claim 21, wherein said N-terminus enhancer group is XQEXEQK (SEQ ID

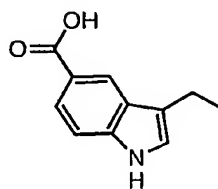
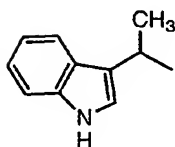
25 NO:38), wherein each X independently is either tryptophan or tryptophan analog.

23. The peptide of claim 21, wherein said C-terminus enhancer group is XPXYVXL (SEQ ID NO:39), or residues 1, 1-2, 1-3, 1-4, 1-5, or 1-6 of SEQ ID NO:39, wherein each X independently is either tryptophan or tryptophan analog.

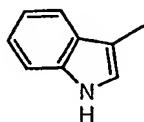
24. The peptides of claims 22-23, wherein each X R group is independently selected from the group
5 consisting of:

Trp(5-CH₂NH₂)

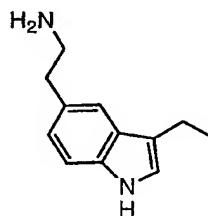
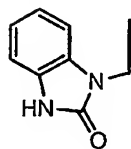
neo-Trp

Trp(5-CO₂H)

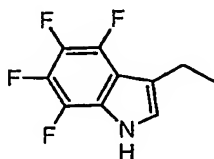
Beta-MeTrp



nor-Trp

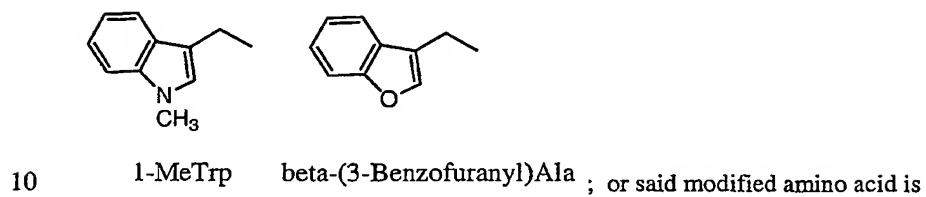
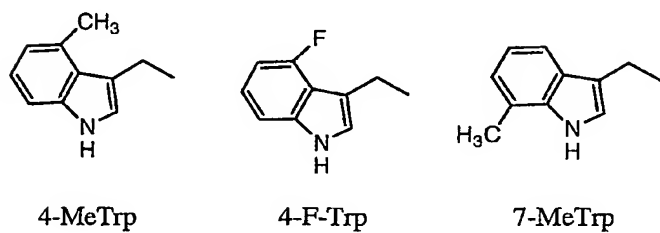
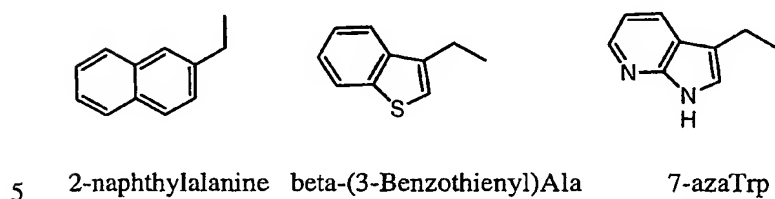
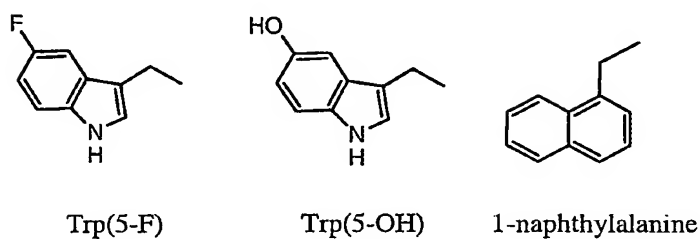
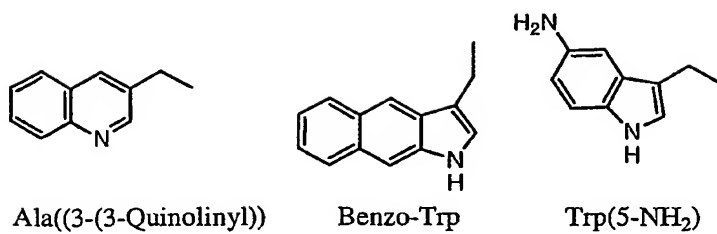
Trp(5-CH₂CH₂NH₂)

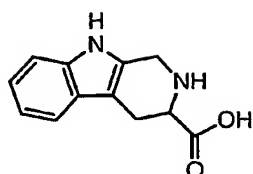
(1-Benzimidazolonyl)alanine



Trp(4,5,6,7-tetrafluoro)

10





1,2,3,4-tetrahydro-9H-pyrido(3,4-b)indole-3-carboxylic acid.

25. The inhibitor of claim 1 wherein the inhibitor has an HR region selected from the HR1 or HR2 sequence of coronavirus, optionally modified with one or more conservative substitutions, and
- 5 is: (a) modified to contain one or more lactam bridges each of which is independently in an (i, i + 3), (i, i + 4) or (i, i + 7) orientation; and (b) optionally modified to contain one or more conservative substitutions.
26. The peptide of claim 25 wherein N-terminus, C-terminus, or both the N-terminus and C-terminus of the peptide contain a protecting group.
- 10 27. The peptide of claim 25, wherein each of said lactam bridges is independently formed between: (a) an amino acid providing a carboxyl moiety, selected from the group consisting of aspartic acid, glutamic acid, 2-aminohexanedioic acid and 2-aminoheptanedioic acid; and (b) an amino acid providing an amino moiety, selected from the group consisting of lysine, ornithine, diaminobutyric acid, and diaminopropanoic acid.

15

1/6

1 MFIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSD
51 TLYLTQDLFLPFYSNVTGFHTINHTFGNPFVIPFKDGIYFAATEKSNVVRG
101 WVFGSTMNNKSQSVIIINNSTNVVIRACNFELCDNPFPAVSKPMGTQTHT
151 MIFDNAFNCTFEYISDAFSLDVSEKSGNFKHLREFVFKNKDGLYVYKGY
201 QPIDVVRDLPSGFNTLKPFIKPLPLGINITNFRAILTAFSPAQDIWGTSA
251 AYFVGYLKPTTFMLKYDENGITITDAVDCSQNPLAELKCSVKSFEIDKGIY
301 QTSNFRVVPSPGDVVRFPNITNLCPFGEVFNATKFPSTVYAWERKKISNCVA
351 DYSLVYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDVVRQIAPG
401 QTGVIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRP
451 FERDISNVFPSPDGKPCPTPPALNCYWPLNDYGFTTTGIGYQPYRVVLS
501 FELLNAPATVCGPKLSTDLIKNQCVNFNFENGLTGTGVLTTPSSKRFQPFQQ
551 FGRDVSDFDTSVRDPKTSEILDISPCAFGGVSVITPGTNASSEVAVLYQD
601 VNCTDVSTAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAEHVDTSYECDI
651 PIGAGICASYHTVSLLRSTSQKSIVAYTMSLGADSSIAYSNNNTIAIPTNF
701 SISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRALS
751 GIAAEQDRNTREVFAQVKQMYKTPTLKYFGGFNFSQILPDPLKPTKRSFI
801 EDLLFNKVTLADAGFMKQYGECLGDINARDLICAQKFNGLTVLPPLLTD
851 MIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYE
901 NQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSS
951 NFGAISSVLNDILSRDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEI
1001 RASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQAAPHGVVFLHVTYV
1051 PSQERNFTTAPAICHEGKAYFPREGVFVFNGTSWFTQNRNFFSPQIITD
1101 NTFVSGNCDVVIGIINNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGD
1151 ISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYVWL
1201 GFIAGLIAIVMTILLCCMTSCCSCLKGACSCGSCCKFDEDDSEPVLKGV
1251 KLHYT

Figure 1

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SARS-HR2	a' d' a' d' a' d' a' d' a' d'	
	ISGINASVVNIQKEIDRLNEVAKNLNESLIDLQEL	(SEQ ID NO:4)
	f b f b f b f b f b	
GCN4	RMKQLEDKVEELLSKNYHLENEVARLKKLVGER	(SEQ ID NO:5)
(Cys) HR2G	Ac-CGGIMKINEDVVEIQLSINYLNENAVLNKKLVGLQ-NH2	(SEQ ID NO:6)
(Cys) GCN4	Ac-CGGRMKQLEDKVEELLSKNYHLENEVARLKKLVGER	(SEQ ID NO:7)

Figure 2

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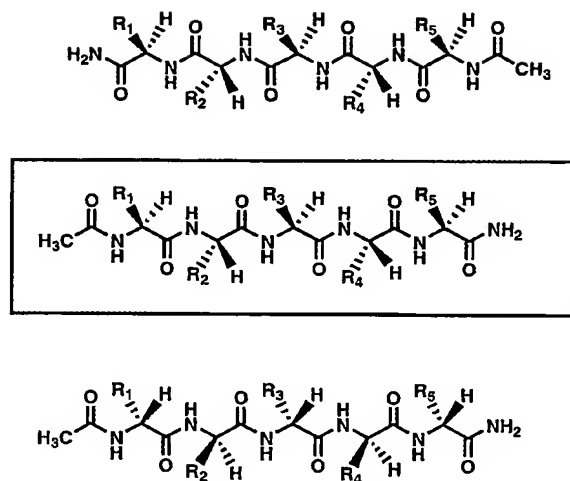
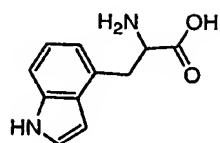
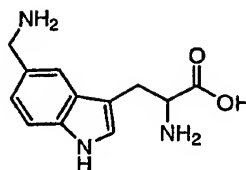
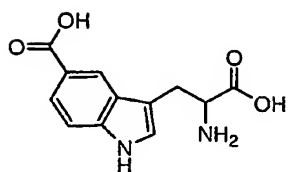
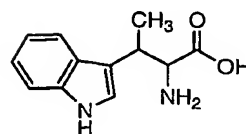


Figure 3

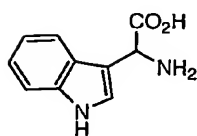
4/6



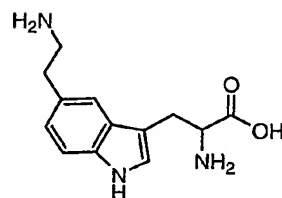
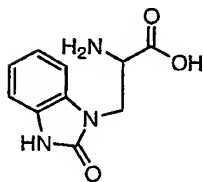
neo-Trp

Trp(5-CH₂NH₂)Trp(5-CO₂H)

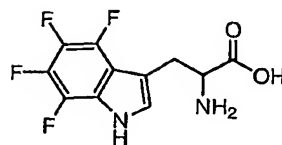
Beta-MeTrp



nor-Trp

Trp(5-CH₂CH₂NH₂)

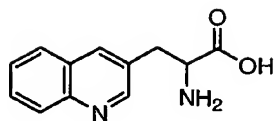
(1-Benzimidazolonyl)alanine



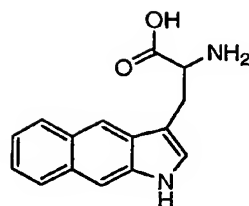
Trp(4,5,6,7-tetrafluoro)

Figure 4A

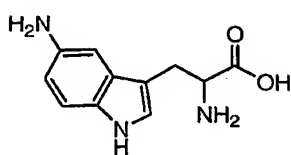
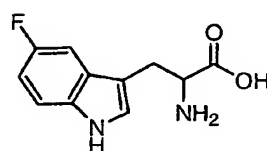
5/6



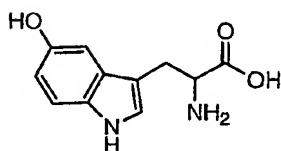
Ala((3-(3-Quinolinyl))



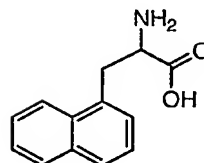
Benzo-Trp

Trp(5-NH₂)

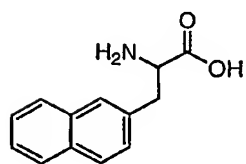
Trp(5-F)



Trp(5-OH)



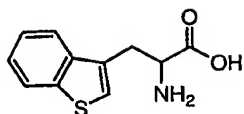
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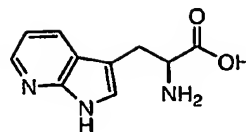
2-naphthylalanine

Figure 4B

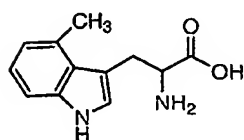
6/6



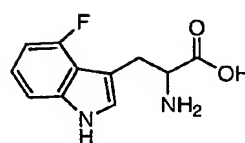
beta-(3-Benzothiényl)Ala



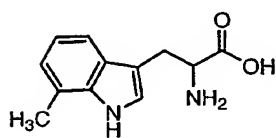
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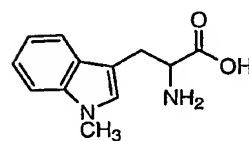
4-MeTrp



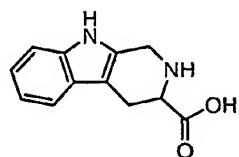
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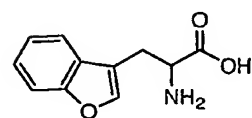
7-MeTrp



1-MeTrp



1,2,3,4-tetrahydro-9H-pyrido(3,4-b)indole-3-carboxylic acid



beta-(3-Benzofuranyl)Ala

Figure 4C

SEQUENCE LISTING

<110> Merck & Co., Inc.
Istituto Di Ricerche Di Biologia Molecolare P. Angeletti S.P.A.

<120> INHIBITORS OF CORONAVIRUS

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<160> 45

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 1255
<212> PRT
<213> SARS-CoV

<400> 1
Met Phe Ile Phe Leu Leu Phe Leu Thr Leu Thr Ser Gly Ser Asp Leu
1 5 10 15
Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala Pro Asn Tyr Thr Gln
20 25 30
His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg
35 40 45
Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser
50 55 60
Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
65 70 75 80
Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
85 90 95
Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln
100 105 110
Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys
115 120 125
Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met
130 135 140
Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr
145 150 155 160
Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser
165 170 175
Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly
180 185 190
Phe Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile Asp Val Val Arg Asp
195 200 205

Leu	Pro	Ser	Gly	Phe	Asn	Thr	Leu	Lys	Pro	Ile	Phe	Lys	Leu	Pro	Leu
	210					215					220				
Gly	Ile	Asn	Ile	Thr	Asn	Phe	Arg	Ala	Ile	Leu	Thr	Ala	Phe	Ser	Pro
225					230					235					240
Ala	Gln	Asp	Ile	Trp	Gly	Thr	Ser	Ala	Ala	Tyr	Phe	Val	Gly	Tyr	
				245					250					255	
Leu	Lys	Pro	Thr	Thr	Phe	Met	Leu	Lys	Tyr	Asp	Glu	Asn	Gly	Thr	Ile
			260					265					270		
Thr	Asp	Ala	Val	Asp	Cys	Ser	Gln	Asn	Pro	Leu	Ala	Glu	Leu	Lys	Cys
	275						280					285			
Ser	Val	Lys	Ser	Phe	Glu	Ile	Asp	Lys	Gly	Ile	Tyr	Gln	Thr	Ser	Asn
	290					295					300				
Phe	Arg	Val	Val	Pro	Ser	Gly	Asp	Val	Val	Arg	Phe	Pro	Asn	Ile	Thr
305					310					315					320
Asn	Leu	Cys	Pro	Phe	Gly	Glu	Val	Phe	Asn	Ala	Thr	Lys	Phe	Pro	Ser
				325					330					335	
Val	Tyr	Ala	Trp	Glu	Arg	Lys	Lys	Ile	Ser	Asn	Cys	Val	Ala	Asp	Tyr
			340					345					350		
Ser	Val	Leu	Tyr	Asn	Ser	Thr	Phe	Phe	Ser	Thr	Phe	Lys	Cys	Tyr	Gly
	355						360					365			
Val	Ser	Ala	Thr	Lys	Leu	Asn	Asp	Leu	Cys	Phe	Ser	Asn	Val	Tyr	Ala
	370					375					380				
Asp	Ser	Phe	Val	Val	Lys	Gly	Asp	Asp	Val	Arg	Gln	Ile	Ala	Pro	Gly
385					390					395					400
Gln	Thr	Gly	Val	Ile	Ala	Asp	Tyr	Asn	Tyr	Lys	Leu	Pro	Asp	Asp	Phe
				405					410					415	
Met	Gly	Cys	Val	Leu	Ala	Trp	Asn	Thr	Arg	Asn	Ile	Asp	Ala	Thr	Ser
				420				425					430		
Thr	Gly	Asn	Tyr	Asn	Tyr	Lys	Tyr	Arg	Tyr	Leu	Arg	His	Gly	Lys	Leu
	435						440					445			
Arg	Pro	Phe	Glu	Arg	Asp	Ile	Ser	Asn	Val	Pro	Phe	Ser	Pro	Asp	Gly
	450					455					460				
Lys	Pro	Cys	Thr	Pro	Pro	Ala	Leu	Asn	Cys	Tyr	Trp	Pro	Leu	Asn	Asp
465					470					475					480
Tyr	Gly	Phe	Tyr	Thr	Thr	Thr	Gly	Ile	Gly	Tyr	Gln	Pro	Tyr	Arg	Val
				485					490					495	
Val	Val	Leu	Ser	Phe	Glu	Leu	Leu	Asn	Ala	Pro	Ala	Thr	Val	Cys	Gly
			500					505					510		
Pro	Lys	Leu	Ser	Thr	Asp	Leu	Ile	Lys	Asn	Gln	Cys	Val	Asn	Phe	Asn
		515					520					525			
Phe	Asn	Gly	Leu	Thr	Gly	Thr	Gly	Val	Leu	Thr	Pro	Ser	Ser	Lys	Arg
	530					535					540				
Phe	Gln	Pro	Phe	Gln	Gln	Phe	Gly	Arg	Asp	Val	Ser	Asp	Phe	Thr	Asp
545					550					555					560
Ser	Val	Arg	Asp	Pro	Lys	Thr	Ser	Glu	Ile	Leu	Asp	Ile	Ser	Pro	Cys
				565					570					575	
Ala	Phe	Gly	Gly	Val	Ser	Val	Ile	Thr	Pro	Gly	Thr	Asn	Ala	Ser	Ser
			580					585					590		
Glu	Val	Ala	Val	Leu	Tyr	Gln	Asp	Val	Asn	Cys	Thr	Asp	Val	Ser	Thr
	595						600					605			
Ala	Ile	His	Ala	Asp	Gln	Leu	Thr	Pro	Ala	Trp	Arg	Ile	Tyr	Ser	Thr
	610					615					620				
Gly	Asn	Asn	Val	Phe	Gln	Thr	Gln	Ala	Gly	Cys	Leu	Ile	Gly	Ala	Glu
625					630					635					640
His	Val	Asp	Thr	Ser	Tyr	Glu	Cys	Asp	Ile	Pro	Ile	Gly	Ala	Gly	Ile
				645					650					655	
Cys	Ala	Ser	Tyr	His	Thr	Val	Ser	Leu	Leu	Arg	Ser	Thr	Ser	Gln	Lys
			660					665					670		
Ser	Ile	Val	Ala	Tyr	Thr	Met	Ser	Leu	Gly	Ala	Asp	Ser	Ser	Ile	Ala
	675						680					685			
Tyr	Ser	Asn	Asn	Thr	Ile	Ala	Ile	Pro	Thr	Asn	Phe	Ser	Ile	Ser	Ile
	690					695					700				

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Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser Val Asp Cys
705      710      715      720
Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ala Asn Leu Leu Leu
725      730      735
Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Ser Gly Ile
740      745      750
Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu Val Phe Ala Gln Val Lys
755      760      765
Gln Met Tyr Lys Thr Pro Thr Lys Tyr Phe Gly Gly Phe Asn Phe
770      775      780
Ser Gln Ile Leu Pro Asp Pro Leu Lys Pro Thr Lys Arg Ser Phe Ile
785      790      795      800
Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly Phe Met
805      810      815
Lys Gln Tyr Gly Glu Cys Leu Gly Asp Ile Asn Ala Arg Asp Leu Ile
820      825      830
Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu Leu Thr
835      840      845
Asp Asp Met Ile Ala Ala Tyr Thr Ala Ala Leu Val Ser Gly Thr Ala
850      855      860
Thr Ala Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile Pro Phe
865      870      875      880
Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr Gln Asn
885      890      895
Val Leu Tyr Glu Asn Gln Lys Gln Ile Ala Asn Gln Phe Asn Lys Ala
900      905      910
Ile Ser Gln Ile Gln Glu Ser Leu Thr Thr Thr Ser Thr Ala Leu Gly
915      920      925
Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn Thr Leu
930      935      940
Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val Leu Asn
945      950      955      960
Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln Ile Asp
965      970      975
Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val Thr Gln
980      985      990
Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser Ala Asn Leu Ala Ala
995      1000      1005
Thr Lys Met Ser Glu Cys Val Leu Gly Gln Ser Lys Arg Val Asp Phe
1010      1015      1020
Cys Gly Lys Gly Tyr His Leu Met Ser Phe Pro Gln Ala Ala Pro His
1025      1030      1035      1040
Gly Val Val Phe Leu His Val Thr Tyr Val Pro Ser Gln Glu Arg Asn
1045      1050      1055
Phe Thr Thr Ala Pro Ala Ile Cys His Glu Gly Lys Ala Tyr Phe Pro
1060      1065      1070
Arg Glu Gly Val Phe Val Phe Asn Gly Thr Ser Trp Phe Ile Thr Gln
1075      1080      1085
Arg Asn Phe Phe Ser Pro Gln Ile Ile Thr Thr Asp Asn Thr Phe Val
1090      1095      1100
Ser Gly Asn Cys Asp Val Val Ile Gly Ile Ile Asn Asn Thr Val Tyr
1105      1110      1115      1120
Asp Pro Leu Gln Pro Glu Leu Asp Ser Phe Lys Glu Glu Leu Asp Lys
1125      1130      1135
Tyr Phe Lys Asn His Thr Ser Pro Asp Val Asp Leu Gly Asp Ile Ser
1140      1145      1150
Gly Ile Asn Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp Arg Leu
1155      1160      1165
Asn Glu Val Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu Gln Glu
1170      1175      1180
Leu Gly Lys Tyr Glu Gln Tyr Ile Lys Trp Pro Trp Tyr Val Trp Leu
1185      1190      1195      1200

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Gly Phe Ile Ala Gly Leu Ile Ala Ile Val Met Val Thr Ile Leu Leu
 1205 1210 1215
 Cys Cys Met Thr Ser Cys Cys Ser Cys Leu Lys Gly Ala Cys Ser Cys
 1220 1225 1230
 Gly Ser Cys Cys Lys Phe Asp Glu Asp Asp Ser Glu Pro Val Leu Lys
 1235 1240 1245
 Gly Val Lys Leu His Tyr Thr
 1250 1255

<210> 2
 <211> 57
 <212> PRT
 <213> SRS-CoV

<400> 2
 Pro Asp Val Asp Leu Gly Asp Ile Ser Gly Ile Asn Ala Ser Val Val
 1 5 10 15
 Asn Ile Gln Lys Glu Ile Asp Arg Leu Asn Glu Val Ala Lys Asn Leu
 20 25 30
 Asn Glu Ser Leu Ile Asp Leu Gln Glu Leu Gly Lys Tyr Glu Gln Tyr
 35 40 45
 Ile Lys Trp Pro Trp Tyr Val Trp Leu
 50 55

<210> 3
 <211> 35
 <212> PRT
 <213> SARS-CoV

<400> 3
 Leu Gly Asp Ile Ser Gly Ile Asn Ala Ser Val Val Asn Ile Gln Lys
 1 5 10 15
 Glu Ile Asp Arg Leu Asn Glu Val Ala Lys Asn Leu Asn Glu Ser Leu
 20 25 30
 Ile Asp Leu
 35

<210> 4
 <211> 35
 <212> PRT
 <213> SARS-CoV

<400> 4
 Ile Ser Gly Ile Asn Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp
 1 5 10 15
 Arg Leu Asn Glu Val Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu
 20 25 30
 Gln Glu Leu
 35

<210> 5
 <211> 33
 <212> PRT
 <213> Yeast

<400> 5
 Arg Met Lys Gln Leu Glu Asp Lys Val Glu Glu Leu Leu Ser Lys Asn
 1 5 10 15

Tyr His Leu Glu Asn Glu Val Ala Arg Leu Lys Lys Leu Val Gly Glu
 20 25 30
 Arg

<210> 6
 <211> 36
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Chimeric sequence of SARS-CoV sequence and yeast
 sequence

<221> ACETYLTATION
 <222> 1

<221> AMIDATION
 <222> 36

<400> 6
 Cys Gly Gly Ile Met Lys Ile Asn Glu Asp Val Val Glu Ile Gln Leu
 1 5 10 15
 Ser Ile Asn Tyr Leu Asn Glu Asn Ala Val Ala Leu Asn Lys Lys Leu
 20 25 30
 Val Gly Leu Gln
 35

<210> 7
 <211> 36
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Modified yeast sequence

<221> ACETYLTATION
 <222> 1

<400> 7
 Cys Gly Gly Arg Met Lys Gln Leu Glu Asp Lys Val Glu Glu Leu Leu
 1 5 10 15
 Ser Lys Asn Tyr His Leu Glu Asn Glu Val Ala Arg Leu Lys Lys Leu
 20 25 30
 Val Gly Glu Arg
 35

<210> 8
 <211> 39
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Modified SARS-CoV sequence

<400> 8
 Cys Cys Gly Thr Thr Thr Ser Thr Ala Leu Gly Lys Leu Gln Asp Val
 1 5 10 15

Val Asn Gln Asn Ala Gln Ala Leu Asn Thr Leu Val Lys Gln Leu Ser
 20 25 30
 Ser Asn Phe Gly Ala Ile Ser
 35

<210> 9
 <211> 32
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Modified SARS-CoV sequence

<400> 9
 Cys Cys Gly Glu Asn Gln Lys Gln Ile Ala Asn Gln Phe Asn Lys Ala
 1 5 10 15
 Ile Ser Gln Ile Gln Glu Ser Leu Thr Thr Thr Ser Thr Ala Leu Gly
 20 25 30

<210> 10
 <211> 41
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Modified SARS-CoV sequence

<400> 10
 Cys Cys Gly Ala Leu Asn Thr Leu Val Lys Gln Leu Ser Ser Asn Phe
 1 5 10 15
 Gly Ala Ile Ser Ser Val Leu Asn Asp Ile Leu Ser Arg Leu Asp Lys
 20 25 30
 Val Glu Ala Glu Val Gln Ile Asp Arg
 35 40

<210> 11
 <211> 33
 <212> PRT
 <213> Yeast

<400> 11
 Arg Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Leu Ser Lys Gln
 1 5 10 15
 Tyr His Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Gly Glu
 20 25 30
 Arg

<210> 12
 <211> 34
 <212> PRT
 <213> Yeast

<400> 12
 Arg Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Glu Ser Lys Gln
 1 5 10 15
 Lys Lys Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Gly Glu
 20 25 30

Arg Tyr

<210> 13
 <211> 15
 <212> PRT
 <213> Yeast

<400> 13
 Lys Ile Lys Lys Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu
 1 5 10 15

<210> 14
 <211> 31
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> derivative of Leucine zipper sequence

<221> ACETYLATION
 <222> 1

<221> AMIDATION
 <222> 31

<400> 14
 Tyr Gly Gly Ile Glu Lys Lys Ile Glu Ala Ile Glu Lys Lys Ile Glu
 1 5 10 15
 Ala Ile Glu Lys Lys Ile Glu Ala Ile Glu Lys Lys Ile Glu Ala
 20 25 30

<210> 15
 <211> 31
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> derivative of isoleucine zipper sequence

<221> ACETYLATION
 <222> 1

<221> AMIDATION
 <222> 31

<400> 15
 Tyr Gly Gly Ile Lys Lys Glu Ile Glu Ala Ile Lys Lys Glu Gln Glu
 1 5 10 15
 Ala Ile Lys Lys Lys Ile Glu Ala Ile Glu Lys Glu Ile Glu Ala
 20 25 30

<210> 16
 <211> 64
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Chimeric sequence including SARS-CoV sequence

<400> 16

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Thr Ile Lys Lys Glu Ile Glu Ala Ile Lys Lys Glu Gln Glu Ala Ile
 1           5           10           15
Lys Lys Lys Ile Glu Ala Ile Glu Lys Glu Ile Thr Thr Thr Ser Thr
          20           25           30
Ala Leu Gly Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu
      35           40           45
Asn Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Gly
 50           55           60

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<210> 17

<211> 68

<212> PRT

<213> Artificial Sequence

<220>

<223> Chimeric sequence including SARS-CoV sequence

<400> 17

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Thr Arg Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Glu Ser Lys
 1           5           10           15
Gln Lys Lys Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Thr
          20           25           30
Thr Thr Ser Thr Ala Leu Gly Lys Leu Gln Asp Val Val Asn Gln Asn
      35           40           45
Ala Gln Ala Leu Asn Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly
 50           55           60
Ala Ile Ser Gly
65

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<210> 18

<211> 66

<212> PRT

<213> Artificial Sequence

<220>

<223> chimeric sequence

<400> 18

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Arg Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Glu Ser Lys Gln
 1           5           10           15
Lys Lys Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Ser Gln
          20           25           30
Ile Gln Glu Ser Leu Thr Thr Thr Ser Thr Ala Leu Gly Lys Leu Gln
      35           40           45
Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn Thr Leu Val Lys Gln
 50           55           60
Leu Ser
65

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<210> 19

<211> 59

<212> PRT

<213> Artificial Sequence

<220>

<223> Chimeric sequence including SARS-CoV sequence

<400> 19
 Arg Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Glu Ser Lys Gln
 1 5 10 15
 Lys Lys Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Glu Asn
 20 25 30
 Gln Lys Gln Ile Ala Asn Gln Phe Asn Lys Ala Ile Ser Gln Ile Gln
 35 40 45
 Glu Ser Leu Thr Thr Thr Ser Thr Ala Leu Gly
 50 55

<210> 20
 <211> 59
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Chimeric sequence containing SARS-CoV sequence

<400> 20
 Thr Arg Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Glu Ser Lys
 1 5 10 15
 Gln Lys Lys Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Glu Asn
 20 25 30
 Gln Lys Gln Ile Ala Asn Gln Phe Asn Lys Ala Ile Ser Gln Ile Gln
 35 40 45
 Glu Ser Leu Thr Thr Thr Ser Thr Ala Leu Gly
 50 55

<210> 21
 <211> 56
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Chimeric sequence containing SARS-CoV sequence

<400> 21
 Thr Ile Lys Lys Glu Ile Glu Ala Ile Lys Lys Glu Gln Glu Ala Ile
 1 5 10 15
 Lys Lys Lys Ile Glu Ala Ile Glu Lys Glu Ile Glu Asn Gln Lys Gln
 20 25 30
 Ile Ala Asn Gln Phe Asn Lys Ala Ile Ser Gln Ile Gln Glu Ser Leu
 35 40 45
 Thr Thr Thr Ser Thr Ala Leu Gly
 50 55

<210> 22
 <211> 49
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Chimeric sequence containing SARS-CoV sequence

<400> 22
 Thr Ile Lys Lys Glu Ile Glu Ala Ile Lys Lys Glu Gln Glu Ala Ile
 1 5 10 15

Lys Lys Lys Ile Glu Ala Ile Glu Lys Arg Leu Gln Ser Leu Gln Thr
 20 25 30
 Tyr Val Thr Gln Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser Ala
 35 40 45
 Asn

<210> 23
 <211> 53
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Chimeric sequence containing SARS-CoV sequence

<400> 23
 Thr Arg Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Glu Ser Lys
 1 5 10 15
 Gln Lys Lys Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Arg Leu Gln
 20 25 30
 Ser Leu Gln Thr Tyr Val Thr Gln Gln Leu Ile Arg Ala Ala Glu Ile
 35 40 45
 Arg Ala Ser Ala Asn
 50

<210> 24
 <211> 67
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Chimeric sequence containing SARS-CoV sequence

<400> 24
 Thr Arg Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Glu Ser Lys
 1 5 10 15
 Gln Lys Lys Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Ala Leu Asn
 20 25 30
 Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val
 35 40 45
 Leu Asn Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln
 50 55 60
 Ile Asp Arg
 65

<210> 25
 <211> 63
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Chimeric sequence containing SARS-CoV sequence

<400> 25
 Thr Ile Lys Lys Glu Ile Glu Ala Ile Lys Lys Glu Gln Glu Ala Ile
 1 5 10 15
 Lys Lys Lys Ile Glu Ala Ile Glu Lys Ala Leu Asn Thr Leu Val Lys
 20 25 30

Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val Leu Asn Asp Ile
 35 40 45
 Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln Ile Asp Arg
 50 55 60

<210> 26
 <211> 62
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> chimeric sequence

<400> 26
 Ile Lys Lys Glu Ile Glu Ala Ile Lys Lys Glu Gln Glu Ala Ile Lys
 1 5 10 15
 Lys Lys Ile Glu Ala Ile Glu Lys Glu Ile Ser Gln Ile Gln Glu Ser
 20 25 30
 Leu Thr Thr Thr Ser Thr Ala Leu Gly Lys Leu Gln Asp Val Val Asn
 35 40 45
 Gln Asn Ala Gln Ala Leu Asn Thr Leu Val Lys Gln Leu Ser
 50 55 60

<210> 27
 <211> 58
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> chimeric sequence

<400> 27
 Ile Lys Lys Glu Ile Glu Ala Ile Lys Lys Glu Gln Glu Ala Ile Lys
 1 5 10 15
 Lys Lys Ile Glu Ala Ile Asn Val Leu Tyr Glu Asn Gln Lys Gln Ile
 20 25 30
 Ala Asn Gln Phe Asn Lys Ala Ile Ser Gln Ile Gln Glu Ser Leu Thr
 35 40 45
 Thr Thr Ser Thr Ala Leu Gly Lys Leu Gln
 50 55

<210> 28
 <211> 62
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Chimeric sequence containing SARS-CoV sequence

<400> 28
 Ile Lys Lys Glu Ile Glu Ala Ile Lys Lys Glu Gln Glu Ala Ile Lys
 1 5 10 15
 Lys Lys Ile Glu Ala Ile Glu Lys Glu Ile Glu Asn Gln Lys Gln Ile
 20 25 30
 Ala Asn Gln Phe Asn Lys Ala Ile Ser Gln Ile Gln Glu Ser Leu Thr
 35 40 45
 Thr Thr Ser Thr Ala Leu Gly Lys Leu Gln Asp Val Val Asn
 50 55 60

<210> 29
 <211> 62
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Chimeric sequence containing SARS-CoV sequence

<400> 29
 Ile Lys Lys Glu Ile Glu Ala Ile Lys Lys Glu Gln Glu Ala Ile Lys
 1 5 10 15
 Lys Lys Ile Glu Ala Ile Glu Lys Glu Ile Asn Gln Asn Ala Gln Ala
 20 25 30
 Leu Asn Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser
 35 40 45
 Ser Val Leu Asn Asp Ile Leu Ser Arg Leu Asp Lys Val Glu
 50 55 60

<210> 30
 <211> 41
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Chimeric sequence containing SARS-CoV sequence

<400> 30
 Ile Lys Lys Glu Ile Glu Ala Ile Lys Lys Glu Gln Glu Ala Ile Lys
 1 5 10 15
 Lys Lys Ile Glu Ala Ile Glu Lys Ser Leu Thr Thr Thr Ser Thr Ala
 20 25 30
 Leu Gly Lys Leu Gln Asp Val Val Asn
 35 40

<210> 31
 <211> 41
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Chimeric sequence containing SARS-CoV sequence

<400> 31
 Ile Lys Lys Glu Ile Glu Ala Ile Lys Lys Glu Gln Glu Ala Ile Lys
 1 5 10 15
 Lys Lys Ile Glu Ala Ile Glu Lys Ala Ile Ser Ser Val Leu Asn Asp
 20 25 30
 Ile Leu Ser Arg Leu Asp Lys Val Glu
 35 40

<210> 32
 <211> 64
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> chimeric sequence

<400> 32
 Ser Gln Ile Gln Glu Ser Leu Thr Thr Thr Ser Thr Ala Leu Gly Lys
 1 5 10 15
 Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn Thr Leu Val
 20 25 30
 Lys Gln Leu Ser Ser Ile Lys Lys Glu Ile Glu Ala Ile Lys Lys Glu
 35 40 45
 Gln Glu Ala Ile Lys Lys Lys Ile Glu Ala Ile Glu Lys Glu Ile Gly
 50 55 60

<210> 33
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> designed linker sequence

<400> 33
 Gly Gly Gly Ser Ser Gly Gly Gly Ser Gly Gly
 1 5 10

<210> 34
 <211> 290
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Chimeric sequence containing SARS-CoV sequence

<400> 34
 Met Val Leu Tyr Glu Asn Gln Lys Gln Ile Ala Asn Gln Phe Asn Lys
 1 5 10 15
 Ala Ile Ser Gln Ile Gln Glu Ser Leu Thr Thr Thr Ser Thr Ala Leu
 20 25 30
 Gly Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gly Gly Gly Ser Ser
 35 40 45
 Gly Gly Gly Ser Gly Gly Asp Ile Ser Gly Ile Asn Ala Ser Val Val
 50 55 60
 Asn Ile Gln Lys Glu Ile Asp Arg Leu Asn Glu Val Ala Lys Asn Leu
 65 70 75 80
 Asn Glu Ser Leu Ile Asp Leu Gln Glu Leu Gly Gly Ser Ser Gly
 85 90 95
 Gly Gly Ser Gly Gly Val Leu Tyr Glu Asn Gln Lys Gln Ile Ala Asn
 100 105 110
 Gln Phe Asn Lys Ala Ile Ser Gln Ile Gln Glu Ser Leu Thr Thr Thr
 115 120 125
 Ser Thr Ala Leu Gly Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gly
 130 135 140
 Gly Gly Ser Ser Gly Gly Ser Gly Gly Asp Ile Ser Gly Ile Asn
 145 150 155 160
 Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp Arg Leu Asn Glu Val
 165 170 175
 Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu Gln Glu Leu Gly Gly
 180 185 190
 Gly Ser Ser Gly Gly Gly Ser Gly Gly Val Leu Tyr Glu Asn Gln Lys
 195 200 205
 Gln Ile Ala Asn Gln Phe Asn Lys Ala Ile Ser Gln Ile Gln Glu Ser
 210 215 220

Leu Thr Thr Thr Ser Thr Ala Leu Gly Lys Leu Gln Asp Val Val Asn
 225 230 235 240
 Gln Asn Ala Gly Gly Gly Ser Ser Gly Gly Ser Gly Gly Asp Ile
 245 250 255
 Ser Gly Ile Asn Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp Arg
 260 265 270
 Leu Asn Glu Val Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu Gln
 275 280 285
 Glu Leu
 290

<210> 35
 <211> 284
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Chimeric sequence containing SARS-CoV sequence

<400> 35
 Met Thr Ala Leu Gly Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln
 1 5 10 15
 Ala Leu Asn Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile
 20 25 30
 Ser Ser Val Leu Asn Asp Ile Leu Ser Gly Gly Gly Ser Gly Gly
 35 40 45
 Gly Ser Gly Gly Asp Ile Ser Gly Ile Asn Ala Ser Val Val Asn Ile
 50 55 60
 Gln Lys Glu Ile Asp Arg Leu Asn Glu Val Ala Lys Asn Leu Asn Glu
 65 70 75 80
 Ser Leu Ile Asp Leu Gln Glu Leu Gly Gly Gly Ser Ser Gly Gly Gly
 85 90 95
 Ser Gly Gly Thr Ala Leu Gly Lys Leu Gln Asp Val Val Asn Gln Asn
 100 105 110
 Ala Gln Ala Leu Asn Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly
 115 120 125
 Ala Ile Ser Ser Val Leu Asn Asp Ile Leu Ser Gly Gly Gly Ser Ser
 130 135 140
 Gly Gly Gly Ser Gly Gly Asp Ile Ser Gly Ile Asn Ala Ser Val Val
 145 150 155 160
 Asn Ile Gln Lys Glu Ile Asp Arg Leu Asn Glu Val Ala Lys Asn Leu
 165 170 175
 Asn Glu Ser Leu Ile Asp Leu Gln Glu Leu Gly Gly Gly Ser Ser Gly
 180 185 190
 Gly Gly Ser Gly Gly Thr Ala Leu Gly Lys Leu Gln Asp Val Val Asn
 195 200 205
 Gln Asn Ala Gln Ala Leu Asn Thr Leu Val Lys Gln Leu Ser Ser Asn
 210 215 220
 Phe Gly Ala Ile Ser Ser Val Leu Asn Asp Ile Leu Ser Gly Gly Gly
 225 230 235 240
 Ser Ser Gly Gly Gly Ser Gly Gly Asp Ile Ser Gly Ile Asn Ala Ser
 245 250 255
 Val Val Asn Ile Gln Lys Glu Ile Asp Arg Leu Asn Glu Val Ala Lys
 260 265 270
 Asn Leu Asn Glu Ser Leu Ile Asp Leu Gln Glu Leu
 275 280

<210> 36
 <211> 284
 <212> PRT

<213> Artificial Sequence

<220>

<223> Chimeric sequence containing SARS-CoV sequence

<400> 36

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Met Gln Ile Asp Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr
 1           5           10           15
Tyr Val Thr Gln Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser Ala
          20           25           30
Asn Leu Ala Ala Thr Lys Met Ser Glu Gly Gly Gly Ser Ser Gly Gly
 35           40           45
Gly Ser Gly Gly Asp Ile Ser Gly Ile Asn Ala Ser Val Val Asn Ile
 50           55           60
Gln Lys Glu Ile Asp Arg Leu Asn Glu Val Ala Lys Asn Leu Asn Glu
 65           70           75           80
Ser Leu Ile Asp Leu Gln Glu Leu Gly Gly Gly Ser Ser Gly Gly Gly
          85           90           95
Ser Gly Gly Gln Ile Asp Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu
          100          105          110
Gln Thr Tyr Val Thr Gln Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala
          115          120          125
Ser Ala Asn Leu Ala Ala Thr Lys Met Ser Glu Gly Gly Gly Ser Ser
          130          135          140
Gly Gly Gly Ser Gly Gly Asp Ile Ser Gly Ile Asn Ala Ser Val Val
          145          150          155          160
Asn Ile Gln Lys Glu Ile Asp Arg Leu Asn Glu Val Ala Lys Asn Leu
          165          170          175
Asn Glu Ser Leu Ile Asp Leu Gln Glu Leu Gly Gly Gly Ser Ser Gly
          180          185          190
Gly Gly Ser Gly Gly Gln Ile Asp Arg Leu Ile Thr Gly Arg Leu Gln
          195          200          205
Ser Leu Gln Thr Tyr Val Thr Gln Gln Leu Ile Arg Ala Ala Glu Ile
          210          215          220
Arg Ala Ser Ala Asn Leu Ala Ala Thr Lys Met Ser Glu Gly Gly Gly
          225          230          235          240
Ser Ser Gly Gly Gly Ser Gly Gly Asp Ile Ser Gly Ile Asn Ala Ser
          245          250          255
Val Val Asn Ile Gln Lys Glu Ile Asp Arg Leu Asn Glu Val Ala Lys
          260          265          270
Asn Leu Asn Glu Ser Leu Ile Asp Leu Gln Glu Leu
          275          280

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<210> 37

<211> 374

<212> PRT

<213> Artificial Sequence

<220>

<223> chimeric sequence

<400> 37

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Met Gly Ile Gly Val Thr Gln Asn Val Leu Tyr Glu Asn Gln Lys Gln
 1           5           10           15
Ile Ala Asn Gln Phe Asn Lys Ala Ile Ser Gln Ile Gln Glu Ser Leu
          20           25           30
Thr Thr Thr Ser Thr Ala Leu Gly Lys Leu Gln Asp Val Val Asn Gln
          35           40           45
Asn Ala Gln Ala Leu Asn Thr Leu Val Lys Gln Leu Ser Gly Gly Gly
          50           55           60

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Ser Ser Gly Gly Gly Ser Gly Gly Leu Gly Asp Ile Ser Gly Ile Asn
 65 70 75 80
 Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp Arg Leu Asn Glu Val
 85 90 95
 Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu Gln Glu Leu Gly Lys
 100 105 110
 Tyr Glu Gln Tyr Ile Lys Gly Gly Ser Ser Gly Gly Gly Ser Gly
 115 120 125
 Gly Gly Ile Gly Val Thr Gln Asn Val Leu Tyr Glu Asn Gln Lys Gln
 130 135 140
 Ile Ala Asn Gln Phe Asn Lys Ala Ile Ser Gln Ile Gln Glu Ser Leu
 145 150 155 160
 Thr Thr Thr Ser Thr Ala Leu Gly Lys Leu Gln Asp Val Val Asn Gln
 165 170 175
 Asn Ala Gln Ala Leu Asn Thr Leu Val Lys Gln Leu Ser Gly Gly Gly
 180 185 190
 Ser Ser Gly Gly Gly Ser Gly Gly Leu Gly Asp Ile Ser Gly Ile Asn
 195 200 205
 Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp Arg Leu Asn Glu Val
 210 215 220
 Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu Gln Glu Leu Gly Lys
 225 230 235 240
 Tyr Glu Gln Tyr Ile Lys Gly Gly Gly Ser Ser Gly Gly Gly Ser Gly
 245 250 255
 Gly Gly Ile Gly Val Thr Gln Asn Val Leu Tyr Glu Asn Gln Lys Gln
 260 265 270
 Ile Ala Asn Gln Phe Asn Lys Ala Ile Ser Gln Ile Gln Glu Ser Leu
 275 280 285
 Thr Thr Thr Ser Thr Ala Leu Gly Lys Leu Gln Asp Val Val Asn Gln
 290 295 300
 Asn Ala Gln Ala Leu Asn Thr Leu Val Lys Gln Leu Ser Gly Gly Gly
 305 310 315 320
 Ser Ser Gly Gly Gly Ser Gly Gly Leu Gly Asp Ile Ser Gly Ile Asn
 325 330 335
 Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp Arg Leu Asn Glu Val
 340 345 350
 Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu Gln Glu Leu Gly Lys
 355 360 365
 Tyr Glu Gln Tyr Ile Lys
 370

<210> 38

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> enhancer region containing tryptophan and / or a tryptophan analog.

<221> MOD_RES

<222> 1, 4

<223> Xaa = independently either tryptophan or a tryptophan analog.

<400> 38

Xaa Gln Glu Xaa Glu Gln Lys
 1 5

<210> 39

<211> 7
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> enhancer region containg tryptophan and / or a tryptophan analog.

 <221> MOD_RES
 <222> 1, 3
 <223> Xaa = independently either tryptophan or a tryptophan analog

 <400> 39
 Xaa Pro Xaa Tyr Val Xaa Leu
 1 5

 <210> 40
 <211> 8
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> enhancer region containg tryptophan.

 <400> 40
 Trp Gln Glu Trp Glu Gln Lys Ile
 1 5

 <210> 41
 <211> 7
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> enhancer region containg tryptophan.

 <400> 41
 Trp Pro Trp Tyr Val Trp Leu
 1 5

 <210> 42
 <211> 42
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> SARS-CoV sequence containg tryptophan and / or a tryptophan analog.

 <221> MOD_RES
 <222> 36, 38
 <223> Xaa = independently either tryptophan or a tryptophan analog

 <400> 42
 Leu Gly Asp Ile Ser Gly Ile Asn Ala Ser Val Val Asn Ile Gln Lys
 1 5 10 15

Glu Ile Asp Arg Leu Asn Glu Val Ala Lys Asn Leu Asn Glu Ser Leu
 20 25 30
 Ile Asp Leu Xaa Pro Xaa Tyr Val Xaa Leu
 35 40

<210> 43
 <211> 35
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> derivative of SARS-CoV sequence

<400> 43
 Pro Asp Val Asp Lys Gly Asp Ile Ser Gly Ile Asn Ala Ser Val Val
 1 5 10 15
 Asn Ile Gln Lys Glu Ile Asp Arg Leu Asn Glu Val Ala Lys Asn Leu
 20 25 30
 Asn Glu Ser
 35

<210> 44
 <211> 35
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> derivative of SARS-CoV sequence

<221> MOD_RES
 <222> 10
 <223> Xaa = Dbu

<400> 44
 Asp Val Asp Leu Gly Glu Ile Ser Gly Xaa Asn Ala Ser Val Val Asn
 1 5 10 15
 Ile Gln Lys Glu Ile Asp Arg Leu Asn Glu Val Ala Lys Asn Leu Asn
 20 25 30
 Glu Ser Leu
 35

<210> 45
 <211> 35
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> derivative of SARS-CoV sequence

<221> MOD_RES
 <222> 12
 <223> Xaa = Orn

<400> 45
 Val Asp Leu Gly Asp Ile Ser Gly Ile Asn Ala Xaa Val Val Asn Ile
 1 5 10 15
 Gln Lys Glu Ile Asp Arg Leu Asn Glu Val Ala Lys Asn Leu Asn Glu
 20 25 30

Ser Leu Ile
35